

1944

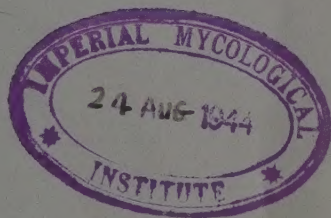
Kentucky Agricultural Experiment Station

University of Kentucky

ANGULAR LEAFSPOT AND WILDFIRE OF TOBACCO

Valleau

BULLETIN 454



Lexington, Kentucky

October, 1943

CONTENTS

	Page
OUTBREAKS AND NAMING OF LEAF DISEASES	3
THE CAUSAL ORGANISMS	6
Effect of Culture Media on the Organisms	6
Colony Types	9
Relationship of the Causal Agents	14
Seed Contamination	18
HOST RANGE OF <i>B. ANGULATUM</i> AND <i>B. TABACUM</i> ...	26
INFECTION STUDIES	28
Sources of Infection	28
Method of Infection of Plants in the Bed	36
Control of Wildfire and Angular Leafspot in the Plant Bed..	38
Inoculation of Tobacco Leaves with <i>Bacterium angulatum</i> and <i>Bacterium tabacum</i>	41
Infection of Water-Soaked Leaves in the Tobacco Field ...	42
Relative Resistance of Tobacco Leaves to Wildfire and Angular Leafspot	44
Susceptibility of Tobacco Plants Under Various Conditions to Leafspot Disease	45
BLACKFIRE	52
SUMMARY	55
LITERATURE CITED	58

Angular Leafspot and Wildfire of Tobacco

By W. D. VALLEAU, E. M. JOHNSON, and STEPHEN DIACHUN

TWO LEAF DISEASES have been destructive to Kentucky tobacco, wildfire (*Bacterium tabacum*) and angular leafspot (*Bacterium angulatum*). This bulletin is a report of studies which have been carried on from time to time at the Kentucky Experiment Station for the past 20 years in an attempt to obtain a clearer understanding of these tobacco leaf diseases, and to find methods which might aid in their prevention. A study of this kind has long been needed, for leafspot diseases of tobacco have been destructive in Kentucky, especially in the dark-tobacco areas, since 1920, and probably previous to that time. In 1924 and 1938 losses over large areas of the dark-tobacco section were estimated conservatively to have ranged up to 60 percent of the crop. In years comparatively free of leafspot, losses which growers considered almost negligible were as high as 20 percent of the crop. Although heavily spotted leaves of dark tobacco were practically valueless, the same degree of injury to burley, especially on flyings and trash, had less influence on the sales price.

OUTBREAKS AND NAMING OF LEAF DISEASES

Wildfire was first recognized as a distinct disease of tobacco in 1917 in North Carolina (50). Since that time it has been found in the majority of tobacco-growing areas of the world. It seems to be fairly characteristic of wildfire that it appears suddenly over an extended area, is destructive for a few years, and then virtually disappears (2). The cause of the sudden appearance and disappearance has not been explained but when it suddenly appears it is usually in an exceptionally wet plant-bed season.

Angular leafspot was recognized as a distinct disease of tobacco following an outbreak in Virginia in 1917 (25). Soon afterwards studies in other tobacco-growing areas in the United States amply confirmed the essential laboratory findings of those in Virginia. In a later Virginia publication (24) the name of the disease was changed from angular leafspot to blackfire, a name which growers had for years applied to a disease of ripening tobacco. No proof was presented that *B. angulatum* caused the large, concentric spot common to and destructive of ripening tobacco, and up to the present no entirely satisfactory explanation of the conditions which bring about this type of

spot has been advanced. Therefore in this report the term angular leafspot is retained for the typical disease produced by *B. angulatum*, and the term blackfire for the disease of ripening dark tobacco. Plants in this advanced stage are nearly immune to infection by *B. angulatum* and *B. tabacum*.

In 1920 angular leafspot was found in many plant beds in Kentucky and in the field where it injured partly-grown burley tobacco. During an extended wet period while tobacco was maturing both burley and dark crops were seriously injured. During late summer a zonate spot developed on burley tobacco at Lexington. Although *B. angulatum* was not consistently isolated from these spots they were recorded as "angular leafspot on ripe leaves of burley tobacco" (43).

Black rust.—In 1921 Houser and Clayton (28) described a disease of cigar tobacco in Ohio, differing from both angular leafspot and wildfire, and called by them "rust" or "black rust." It began on the lower leaves as irregular red spots which increased in size and number, and gradually spread up the plant. Apparently black rust was favored by rainy weather because, as these authors claim, "the timely advent of sunshiny weather arrested its development." When weather favored the disease the "only remedy was prompt harvesting otherwise the entire crop will be ruined." This disease was believed to be a physiological breakdown associated with certain soil and weather conditions. From the description of the disease and the conditions under which it developed it seemed that it might be similar to the late-season disease of dark tobacco in Kentucky and neighboring states.

Leaf spotting.—In 1927, in soil pot culture studies at the Kentucky Experiment Station, a physiological spotting of tobacco developed in the greenhouse somewhat similar to the late-season disease of dark tobacco in the field. As a result of a further study of the former type of spotting, which was repeatedly produced in the absence of pathogenic organisms, and as a result of frequent failure to obtain pathogenic bacteria from the advancing edges of what appeared to be similar spots on field-grown tobacco, the hypothesis was formulated (42) that two kinds of spots were involved in leaf-spot outbreaks—angular leafspot on vigorously growing plants and a physiological spotting on maturing tobacco. It now appears probable that the 1927 type of spot in the greenhouse was a manifestation of phosphorus deficiency (34).

Possibly the frequent failure to obtain bacteria from the con-

centric field spots by the plating method resulted from the fact that bits of tissue from the edges of the spots were cultured, rather than tissue from slightly older portions where the bacteria sometimes appeared to be present in limited areas. When whole spots were either crushed in water or put into broth and incubated and the fluid used for inoculation, the percentage of inoculations which produced leafspot was higher; but this did not prove that the concentric spot was directly caused by the bacteria.

Opinions on causes of spotting.— One of the authors, from 1936 through 1939, studied dark-tobacco leaf diseases, principally in Todd county. He tentatively concluded that most of the late-season leaf spotting in that vicinity, other than the frequently quite destructive mosaic burn, was caused by *B. angulatum*, *B. tabacum*, or both. This conclusion is supported by the fact that plant-bed treatment with bordeaux mixture proved very effective in three seasons in preventing the late-season type of spot on most of the farms where it was tried, and in the fourth season until about August 1, after which bacterial infection gradually built up and blackfire caused very extensive damage (29).

Clayton (12, 14, 15) and more recently J. Johnson (31) have attempted to explain the so-called blackfire type of spotting as an infection in areas of the leaf which remain water-soaked for 24 to 48 hours following hard, driving rain. While there appears to be no doubt that severe injury may result to water-soaked leaves subsequently inoculated with one or the other of these pathogenic organisms, the type of injury pictured in their illustrations is not sufficiently like the field disease, as it occurs annually in western Kentucky, to convince one that a final answer has been given to the question as to how these spots are actually caused under field conditions. There appears to be no question that both the internal and the external types of water-soaking contribute largely to the spread of typical wildfire and angular leafspot.

Conflicting statements about leafspot and wildfire made largely on the basis of observation naturally were a problem of the study. Some of them are enumerated here to indicate the scope of this undertaking. In other sections of the bulletin a more complete report appears. The simple explanation that infection in the plant bed was caused from contaminated seed was not borne out by these studies. The explanation that infection was caused from bacteria which overwinter in tobacco trash (3, 7) or in leaves of other plants (1) subsequently blown into plant beds was not accepted but was diffi-

cult to disprove. Many investigators have arrived at the conclusion that the bacteria cannot overwinter in the soil in spite of much observational evidence to the contrary. From time to time it has been said that high fertility, especially high available nitrates, and rapid growth favor both typical angular leafspot and wildfire, while an unbalanced fertility or even low available nutrients, particularly potassium, have been said to favor the after-topping disease of dark tobacco. These statements, based on observations largely, have added to the difficulties of arriving at a clear understanding of the diseases.

THE CAUSAL ORGANISMS

Effect of Culture Media on the Organisms

One phase of these studies consisted in learning better methods of handling the leafspot organisms in culture. With respect to the wildfire organism, Johnson and Murwin (32) concluded that "the best culture medium for *Bacterium tabacum* seems to be potato-dextrose agar, with transfers at intervals of somewhat less than one month." They kept all cultures between 8° and 10° C, and concluded that the "virulence of the organisms showed a general downward tendency on all media with increasing age, most marked on beef-peptone agar and least marked on potato-dextrose agar." Loss of virulence was reported as complete on beef-peptone agar in 15 months. Presumably loss of virulence meant inability of living organisms, capable of being transferred successfully, to cause infection in tobacco plants. Cultures of *B. tabacum* lost their pathogenicity rapidly, according to Moore (36), when kept on the usual laboratory media. She did not state whether loss of pathogenicity resulted from death of the organism or from inability to cause infection. The organism kept better, she concluded, when stored in a water suspension. Manil (35) found no loss of virulence of *B. tabacum* after a year on meat-broth agar (12 transfers).

In the present studies, no observable loss of virulence (inability to produce typical spotting) occurred, but cultures of *B. angularatum* and *B. tabacum* kept on potato agar plus 2-percent dextrose at room temperature, frequently died in as short a period as 4 days. Therefore the longevity of the organisms was studied on media at room temperature to determine the probable cause of death.

Potato-dextrose (2 percent) agar.—Both organisms grew well on potato agar plus 2-percent dextrose but cultures in plates usually died after 7 or 8 days (Table 1). Death was associated with a rapid increase of hydrogen ions in the medium, from pH 5.6 to 5.8 at the

TABLE 1.—EFFECT OF CULTURE MEDIA ON LONGEVITY OF *B. angularum* AND *B. tabacum*
(Figures indicate percent of streaks on agar plates which were viable.)

Media	Initial pH	Organism	Percent of viable streaks when cultures were streaked at									Final pH
			2-5 days	6-10 days	11-15 days	16-20 days	21-30 days	31-50 days	51-100 days	122 days	300 days	
Potato-dextrose (2 percent) agar	5.6	<i>B. tabacum</i> 2 strains	90	31	5	0	0	—	—	—	—	4.0-4.4
		<i>B. angularum</i> 4 strains	80	24	10	0	0	—	—	—	—	4.0-4.4
Potato-dextrose (2 percent) agar + KOH	7.0	<i>B. angularum</i> 1 strain	—	100	70	0	—	—	—	—	—	4.2-4.6
Potato-dextrose (2 percent) agar + CaCO ₃	7.0	<i>B. angularum</i>	—	—	—	—	—	100	100	—	—	6.4-7.0
Potato-dextrose (2 percent) agar + KH ₂ PO ₄ + KOH		<i>B. tabacum</i>	—	—	—	—	—	100	—	—	—	6.4-7.0
		<i>B. angularum</i> 1 strain	—	100	100	100	33	0	—	—	—	
Potato-dextrose (2 percent) agar + KH ₂ PO ₄		<i>B. angularum</i> 4 strains	—	50	0	—	—	—	—	—	—	
Potato-dextrose (2 percent) agar + KH ₂ PO ₄		<i>B. tabacum</i> 2 strains	—	33	0	—	—	—	—	—	—	
Potato-agar	5.8	<i>B. angularum</i> 4 strains	—	56	64	25	41	50	85	—	—	7.8-8.2
		<i>B. tabacum</i> 1 strain	—	100	75	75	75	50	60	—	—	8.0
		<i>B. angularum</i>	—	—	—	—	—	100	—	—	—	6.8-7.0
		<i>B. tabacum</i>	—	—	—	—	—	100	—	—	—	6.8-7.0
Potato-dextrose (½ percent) agar	5.6		—	—	—	—	—	—	—	—	—	
Beef-peptone broth	7.0	<i>B. angularum</i>	—	—	—	—	—	—	—	—	100	7.0-7.4
		<i>B. tabacum</i>	—	—	—	—	—	—	—	—	100	7.0-7.4
Beef-peptone agar	7.0	<i>B. angularum</i>	—	—	—	—	—	—	—	—	100	6.0-6.6
		<i>B. tabacum</i>	—	—	—	—	—	—	—	—	100	6.0-6.6

beginning of the test to pH 4.4 to 5.2 in 7 days, and pH 4 to 4.4 in 12 days. Potato-dextrose agar was an unsatisfactory medium at room temperature except where the organism was expected to live only a few days, as in isolation work.

Potato-dextrose (2 percent) agar neutralized.—When the potato-dextrose agar was adjusted to neutrality with KOH, the longevity of *B. angulatum* was correspondingly increased in both plates and tubes to about 14 days (Table 1). In tubes the pH ranged from 4.2 to 5.4 in 8 days. In plates it ranged from 5.2 to 5.4 in 7 days and 4.2 to 4.6 in 12 days.

When a liberal quantity of CaCO_3 (.3 g per 20 cc of medium) in the form of precipitated chalk was added to potato-dextrose agar tubes, inoculated with *B. angulatum*, and poured into plates, the organism was living 46 days later, when the plates had dried out (Table 1). The medium changed in 7 days from neutral or very slightly acid to pH 5.2 or below. *B. angulatum* was still alive after 84 days in each of 10 tubes. The reaction after 46 days was between pH 6.4 and 7.

The addition of 1 g monopotassium phosphate and 1 cc 0.1 *N* KOH per tube increased the longevity of *B. angulatum* in tubes. When only 1 g of monopotassium phosphate was used in culture dishes, both organisms died before ten days.

Potato agar.—Potato agar without dextrose became alkaline rather rapidly when inoculated with either organism (Table 1). A marked increase in alkalinity had an injurious effect especially in culture dishes, but the organisms were still alive after 95 days.

Potato-dextrose ($\frac{1}{2}$ percent) agar.—Because of the results obtained with potato-dextrose (2 percent) agar and potato agar, a potato-dextrose ($\frac{1}{2}$ percent) agar was tried. Both organisms grew well on it. Both *B. angulatum* (11 tubes) and *B. tabacum* (12 tubes) changed the first pH reaction from 5.6 to about 7.0 in periods of 49 and 38 days respectively. The cultures were all alive at the end of this period. It is evident that potato agar containing $\frac{1}{2}$ percent dextrose is a more satisfactory medium for routine work with these organisms at room temperature than the usual 2-percent dextrose agar.

Beef-peptone broth.—*B. angulatum* and *B. tabacum* both produced infection on tobacco up to and including about 300 days in beef-peptone broth at room temperature, when the test was terminated, and after 3 years and 6 months when kept at about 5° C. No difference was noted in the effect of the two organisms on the medium,

both tending to increase alkalinity slightly to about pH 7.4. Beef-peptone broth is therefore an excellent medium for routine work and for storing these organisms.

Beef-peptone agar.—Four cultures of *B. angulatum* were viable on beef-peptone agar after 122 days, at which time the agar had dried out. The pH of the agar in the various tubes ranged from 6.0 to 6.6 at the end of the test. Beef-peptone agar appears to be a satisfactory medium at room temperature for these organisms. At 5° C 11 of 14 cultures of *B. angulatum* and 2 of 8 cultures of *B. tabacum* were viable after 3 years and 6 months.

It seems probable that death of the leafspot bacteria on potato-dextrose (2 percent) agar is due to rapid increase in acidity of the medium. A culture medium which remains slightly acid or becomes only slightly alkaline seems to be necessary to prolong the life of the bacterial leafspot organisms in culture.

Water and plain agar.—Moore (36) used sterile water as a medium for storing organisms because of difficulty in maintaining cultures on ordinary media. This method was tried by the writers. Tubes of sterile tap water and of distilled water from 3 different stills were used, keeping them in the refrigerator. At the end of 156 days 4 tap-water cultures were alive, 4 cultures in water from a quartz still were alive, while 1 of 3 in water from another still was alive, but 4 cultures in water from the third still were dead. After 3 years, 9 months and 24 days 3 of 4 cultures of *B. tabacum* in water from 3 stills and 1 of 2 cultures in tap water were viable; while 5 of 6 cultures of *B. angulatum* in water from 3 stills were alive. Cultures were also stored on plain agar. After 3 years and 6 months, 19 of 21 cultures of *B. angulatum* and 5 of 6 cultures of *B. tabacum* were viable.

These studies suggest that loss of virulence of the organism (ability to produce typical spots) is not lost in culture so long as conditions are maintained favorable to continued life. The results also made possible a study of colony types in which a longer period of growth is desired than is possible on ordinary potato-dextrose (2 percent) agar.

Colony Types

Elliott (22) stated that colonies of *B. angulatum* have undulate margins and opaque centers while colonies of *B. tabacum* have smooth margins. Stapp (41) illustrated differences between colonies of different strains of *B. angulatum* and *B. tabacum*. Wsorrow (51) studied a disease of tobacco in Caucasia which he considered similar to that caused by *B. mellium*. Because of the wide range of colony types the organism was named *Phytomonas heterocea*.

In attempting to find some constant difference between the colonies of *B. angulatum* and *B. tabacum* in the present studies it was found that, in thinly sown plates of agar, colonies in a culture prepared from a single leaf spot differed. When the differing colonies were isolated and transferred serially in thinly sown plates the differences were usually perpetuated. In this way many cultures of both *B. tabacum* and *B. angulatum*, each with a distinct colony type, were isolated.

Variation in colony types.—Colonies produced by different strains of *B. angulatum* and *B. tabacum* could be described as watery, flat, flowing; or round, raised, smooth, and watery; or cheesy and smooth; or tough and somewhat leathery. Their shapes were irregular, due to flowing; amoeboid; or round with smooth, feathery, irregular or undulate margins. Their surfaces were flat and smooth, raised and smooth, raised and pushed up as though all growth were taking place in the center; or they were folded into patterns which were distinct for the different strains (Fig. 1).

From the studies made, it would seem that the number of distinct colony types of each species which can be isolated and perpetuated is unlimited. An attempt to distinguish the species on a basis of colony characters was unsuccessful.

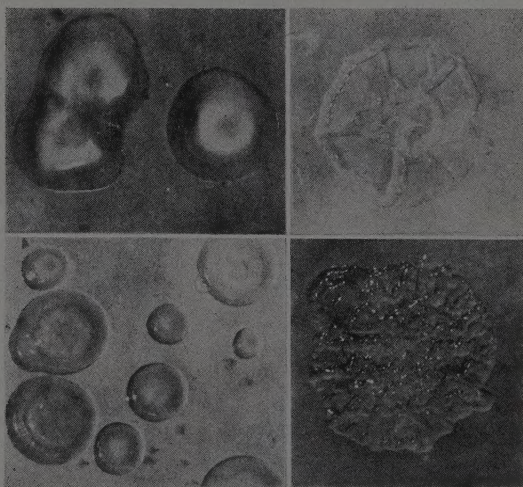


Fig. 1.—Strains of *B. angulatum* illustrating differences in colony characters. The same kind of differences are shown by strains of *B. tabacum*.

Pathogenicity of rough and smooth types.—Inoculations of burley tobacco were made with most of the strains of both organisms isolated. No marked difference, because of roughness or smoothness of colonies, was observed in pathogenicity of any of the strains when inoculations were made on plants in the greenhouse.

Stability of strains in culture media.—Eleven strains of *B. angulatum* differing in colony type were carried through a series of 10 plate transfers to study the stability of type. From each plate a typical colony was selected for seeding the next plate. The colonies in the tenth plate were compared with a plate seeded from the broth culture from which the first plate was seeded. Nine distinct strains of *B. angulatum* showed no change in the 10 platings. Where change appeared to have taken place in 2 strains, the broth cultures also contained more than one type of colony.

Fourteen strains of *B. tabacum* differing in colony type remained relatively constant through 10 plate transfers as compared with plates inoculated from the original tube. One plate contained opaque and watery colonies but both were found in the original tube. One changed from coarsely wrinkled to dense, smooth, raised, but the original tube was found to contain both types.

Single-colony strains of *B. angulatum* and *B. tabacum* were kept in test tubes of distilled water, tap water, and beef-peptone broth from 1933 until December 1937 when inoculations were made on tobacco plants in the greenhouse. Isolations were then made with thin seeding on culture dishes of potato-dextrose ($1\frac{1}{2}$ percent) agar. On this medium there were some differences between colonies of the same strain stored on different media but the colony types of a given strain were remarkably uniform. An occasional plate contained 2 or 3 colony types.

Stability of strains in passage through tobacco leaves.—Ten successive passages of 7 culturally distinct strains of *B. angulatum* were made through burley tobacco leaves. The cultures were designated watery opaque; cheesy opaque; thin bluish; coarsely wrinkled; zonate rough; thin irregular margin; and watery irregular margin. A single plant was used for each strain. When infection was apparent on an inoculated leaf, a bit of infected tissue from it was crushed in water and atomized on a leaf higher on the plant. After the tenth inoculation, a single spot was plated from each plant and the colonies compared with the original culture. Five of the 7 strains showed no change. Two strains, cheesy opaque and zonate rough, separated into cheesy opaque and wrinkled, and zonate rough and

TABLE 2.—COLONY TYPES OF *B. angulatum* AND *B. tabacum* FOUND IN CULTURE DISHES THINLY SEEDED FROM SINGLE LEAFSPOTS COLLECTED FROM THE FIELD

Plate number and source	Colony type	Inoculation test
Plate I (spot collected on Station farm, July 21, 1934)	1. Bluish, smooth, glistening, finely granular 2. Small, white, opaque, smooth 3. Large, rough and folded	Negative Angular leafspot Angular leafspot
Plate II (spot collected from dark tobacco on Station farm, July 19, 1934)	1. Smooth, spreading, irregular margin 2. Circular, raised, small 3. Circular, raised, dense, white, slightly rough 4. Dense, white, large, wrinkled	Angular leafspot Angular leafspot Angular leafspot Angular leafspot
Plate III (spot collected from burley tobacco on Station farm, July 18, 1934)	1. Dense, white, circular, smooth 2. Small, circular, raised in center, rough 3. Small, white, circular, zonate	Angular leafspot Angular leafspot Angular leafspot
Plate IV (spot collected from dark tobacco from Murray, Ky., July 19, 1934)	1. Circular, white, granular 2. Small, circular, white, raised, rough 3. Flat, smooth, zonate, denser in center 4. Circular, bluish center, smooth, white 5. Circular, thin, bluish	Wildfire Wildfire Wildfire Wildfire Wildfire
Plate V (10 spots collected near Murray, Ky., June 14, 1934)	1. Watery, bluish, amoeboid 2. Bluish, flat, wet, center opaque 3. Very rough, wrinkled 4. Smooth, glistening, bluish (separated into circular and amoeboid types) 5. White dryish, raised, very rough and wrinkled. (Separated into wrinkled; smooth; and dense, opaque, feathery margin types) 6. Transparent, depressed centers, dry, circular, coarsely wrinkled 7. Raised, circular, dry, transversely folded 8. Very dry, cheesy, large, white, fine wrinkled, radiating from center 9. Smooth, white, raised, small, gray center, smooth or with crater in center	Wildfire Wildfire Wildfire Wildfire Wildfire Wildfire Wildfire Wildfire Wildfire

smooth, respectively. The original tube of the cheesy opaque strain was found to contain both opaque and wrinkled when plated. These experiments proved that there is a high degree of stability of colony types in passage through plates or a series of tobacco leaves.

Colony types in nature.—Dilution plates were made directly from individual leafspots collected from a plant bed shortly after the first appearance of leafspot in the bed. These were studied for colony differences by thinly sowing plates and by examining the colony types which developed. Striking differences in colonies within a plate were found. The different strains were transferred and remained relatively stable in colony characters in successive transfers. In Table 2 are given the results obtained when colonies were selected from plates thinly seeded from wildfire and angular leafspots collected in the field.

It may be concluded from these studies that in nature numerous strains of *B. angulatum* and *B. tabacum* exist, ranging from smooth, watery colonies through all degrees of roughness, and that these strains may be considered relatively stable. If colonial variations are in part dependent upon differences in capsular condition of the bacteria, then these two organisms retain their pathogenicity through all the range of capsular conditions.

Comparative pathogenicity of strains.—During the progress of the studies on leafspot diseases, numerous cultures of *B. angulatum*



Fig. 2.—Extremes in virulence of single-colony cultures of *B. tabacum* (left) and *B. angulatum* (right). Inoculated Feb. 29, 1936 and Mar. 12, 1936; photographed Mar. 6, 1936 and Mar. 20, 1936, respectively.

and *B. tabacum* have been collected from various parts of the state. To determine the relative virulence of the strains comparative inoculations were made between one culture used consistently on one side of each leaf and a different culture on the other side. By this means the more virulent and less virulent cultures of both organisms were gradually selected. In Fig. 2 are shown the extremes in virulence found in over 36 strains of *B. tabacum* and over 130 strains of *B. angulatum*. Intermediate degrees between these extremes were represented in the cultures studied. Only cultures which produced an appreciable amount of toxin were considered to be *B. tabacum* and only cultures which had originated from typical angular leafspots were considered to be *B. angulatum*.

From these studies it may be concluded that numerous relatively stable strains differing in pathogenicity as well as in cultural characteristics are involved in outbreaks of these diseases.

Relationship of the Causal Agents

Wildfire and angular leafspot undoubtedly are two distinct diseases of tobacco. The critical work of Stapp raised the question whether the diseases were caused by distinct species of bacteria or by the same species. The studies of Braun (9) and those reported in the present paper show that there are many strains of each of the supposed species and these form a rather complete series in symptom expression between nearly nonpathogenic strains of *B. angulatum* and highly pathogenic strains of *B. tabacum*. The only real difference in the two organisms appears to be the ability of *B. tabacum* to produce a *chlorolytic* toxin capable of destroying chlorophyll while *B. angulatum* either does not produce it or produces it only in very small quantities. In the field the wildfire organism appears sporadically in areas where the angular leafspot organism is already established, and seems to disappear as suddenly.

Reid and his co-workers (39, 40) have assumed that because there are antigenic similarities between the common soil saprophyte *Pseudomonas fluorescens* and the wildfire and angular leafspot organisms the three organisms are identical, and that the latter organism may be found on vegetation, such as clover and healthy tobacco leaves, as an epiphyte. They have, however, given no proof that the organisms which they consider from serological tests to be the wildfire and angular leafspot organisms, can produce the respective diseases. Until that is done, it would seem that the organisms should be

differentiated on the basis of the diseases which they cause. A further statement on Reid's paper is given in the footnote.¹

Evidence is gradually accumulating tending to prove that *B. angulatum* and *B. tabacum* are strains of the same species and that one changes into the other. From seed infection studies made in the spring of 1934 in which some seed heads were inoculated with *B. tabacum* and others with *B. angulatum* came our first evidence that one species changed into the other. During the winter, bits of seed pods from the heads inoculated several times with *B. tabacum* gave only wildfire when used as inoculum, but later in the spring only angular leafspot developed when bits of seed pods from the same seed heads were used as inoculum. In 1937 Braun (9) showed that typical angular leafspot may be produced by certain strains of *B. tabacum* arising in culture on potato-dextrose agar transferred frequently over a 1½ year period and kept at 8° to 10° C.

¹ From a serological study of *Pseudomonas fluorescens* and the organisms associated with wildfire and angular leafspot, Reid, et al (39) concluded that the nonpathogenic organism and the two pathogenic organisms of these diseases are identical antigenically, and are also identical with *Phytophthora primulae* and *Ph. cerasi* and possibly with *Ph. vignae* and *Ph. syringae*. These authors state that "serological techniques measure the sum of the morphological and physiological characteristics (including pathogenicity) of the germ plasma of bacterial cells." On the basis of serological tests they "prove" that all the plant-bed soils in their tests contained the wildfire organism and that organisms found on clover and other healthy plants were also the wildfire organism. Failure of the disease to develop in plant beds and in the field in the presence of the organism was attributed to some other factor or factors than absence of the causal agent.

There is a question as to whether the authors were at all justified in concluding that organisms which have been demonstrated to be closely similar by antigenic tests are therefore identical. There seems to be little question that their studies prove a close similarity between *Pseudomonas fluorescens* and *Phytophthora tabaci*, but the organisms cannot be considered identical unless it can be shown that in some phase *Ps. fluorescens* will produce the typical wildfire disease on tobacco. The authors claim, "at frequent intervals strains identified by means of the agglutination technique were tested on susceptible plants as a further check on the identity of the organisms," but nowhere in the paper do they give the results of these tests. In fact, in drawing conclusions as to the presence or absence of the wildfire organism in plant-bed soils and on plants other than tobacco which are not diseased, it is stated that the identifications were on the basis of serological tests. It would have been simpler and far more conclusive if simple inoculation tests had been made to prove pathogenicity rather than to assume pathogenicity from serological tests. The plant pathologist, whether rightly or wrongly, frequently bases species identification on pathogenicity to a given host. He has great difficulty, therefore, in accepting the conclusion that an organism which is similar in certain biological reactions to another is therefore identical with the pathogenic organism.

According to the authors' conclusions, *Ph. tabaci* and *Ph. angulata* are identical, yet the two organisms produce distinct diseases, and *Ph. angulata* is frequently found year after year on farms where *Ph. tabaci* is never found. This is not in accord with the statement that the "organisms of wildfire and angular leafspot are widely distributed in nature and occur normally on many plants as harmless saprophytes." If this were true, we would expect to find wildfire commonly distributed in burley plant beds and fields where heavy infection of angular leafspot occurs, but this is not the case.

(Footnote continued on page 16)

TABLE 3.—RESULTS OF INOCULATIONS TO BURLEY TOBACCO WITH SINGLE COLONY CULTURES OF *B. tabacum* AND *B. angulatum* THAT HAD BEEN STORED AT ICE-BOX TEMPERATURES IN VARIOUS MEDIA

Species	Medium	Age		Results of inoculation
		Years	Days	
<i>B. tabacum</i>	Beef broth	3	185	Angular leafspot
<i>B. angulatum</i>	Distilled water	3	294	Angular leafspot + wildfire
<i>B. angulatum</i>	Distilled water	3	294	Angular leafspot + wildfire
<i>B. angulatum</i>	Distilled water	3	294	Wildfire
<i>B. tabacum</i>	Beef-peptone agar	3	181	Angular leafspot + wildfire
<i>B. tabacum</i>	Beef-peptone agar	3	181	Angular leafspot + wildfire
<i>B. tabacum</i>	Beef-peptone agar	3	181	Angular leafspot

In these studies, inoculations were made to burley tobacco in 1938 with single-colony cultures of *B. tabacum* and *B. angulatum* that had been stored at icebox temperature for over 3 years in broth, distilled water and beef-peptone agar. In Table 3 are shown the results of these inoculations.

All cultures had been tested repeatedly in inoculations prior to storage in the various media, and all but one gave symptoms typical for the species. One *B. angulatum* culture, isolated from seedling plants in a bed visably free of wildfire in an area where wildfire was rare, sometimes caused wildfire and sometimes angular leafspot. In addition, 19 single-colony cultures of *B. angulatum* and 5 of *B. tabacum* stored 3 years and 181 days on plain agar gave symptoms typical of the respective species when inoculations were made to burley tobacco.

(Footnote continued from page 15)

The geneticist recognizes that plants and animals which appear identical and react identically to serological tests may differ, by one or more genetic factors, sufficiently to separate them as distinct entities. It is questionable whether the bacteriologist has any sound basis for the claim of identity when the test used is one which, at best, is generally accepted as showing only close relationship between organisms, or identity between certain constituents of organisms, but is not recognized as a test for complete genetic identity. The fact that some bacteriologists, as a matter of convenience, or sometimes of necessity, use the antigenic reaction as the final word on identification does not prove the assumption that it is a satisfactory test. Until it can be proved that the organisms commonly found on tobacco, clover, and other crops will actually cause wildfire, the plant pathologist cannot accept the identification, as he is primarily interested in the disease-producing organisms and not in the closely similar saprophytes. It may eventually be proved that the wildfire and angular leafspot organisms, as well as certain other plant pathogens, are mutants, variants, adaptations, or phases of *Ps. fluorescens* but until it is proved that *Ps. fluorescens* readily changes to the pathogenic organisms the assumption of the potential danger from *Ps. fluorescens* cannot be accepted. If it is proved that such a change does occur, then the matter of frequency of change will be of prime importance. Present indications from field observations are that, in certain soil areas where angular leafspot is common, the change to the wildfire organism rarely occurs.

Further tests of some of the segregating cultures reported in the preceding table were made. From leaves inoculated with two strains of *B. angulatum* and one strain of *B. tabacum*, on each of which were produced both angular leafspots and wildfire, isolations were made from both kinds of spots. Inoculations from these 6 plates gave the same kind of spot as that from which the isolation was made. Then 10 plates were made from 10 single colonies of each of the 6 isolations and the 60 strains were run through 10 successive plates, selecting a single colony for sowing each time. Inoculations were then made to tobacco from each plate and in every case the expected result was obtained; that is, plates from an original angular leafspot gave angular leafspot and from a wildfire spot gave only wildfire. These results would tend to show that up to this point the cultures should be considered pure.

From the tenth plate of each of the 60 strains a single colony was transferred to broth and stored for 22 months at 40° F, after which inoculations were again made. From 10 tubes of *B. angulatum* tracing back to an original *B. angulatum* isolation, 9 gave angular leafspot and 1 wildfire. Of the 10 wildfire cultures of the same origin all gave wildfire. The second strain of *B. angulatum* gave no evidence of further change as only angular leafspot developed from 10 tubes and only wildfire from the other 10. From 10 *B. angulatum* cultures stemming from an original *B. tabacum* culture, 8 gave wildfire, 1 angular leafspot and one gave no infection. All 10 *B. tabacum* cultures produced wildfire. These results tend to prove that the change in toxigenic properties can proceed in either direction.

There is always a danger of mixture of strains in bacterial cultures although with the organisms under consideration the characteristic appearance of the colonies tends to lessen the danger of mixtures if single-colony transfers are made. To eliminate danger of mixture, 54 single-cell isolations were made from 2 single-colony strains of *B. angulatum* and 65 from 2 single-colony strains of *B. tabacum*. These cultures were stored in beef broth at 40° F. At intervals inoculations were made to tobacco. Up to and including 1,142 days, no change had occurred in any of the 54 strains of *B. angulatum*. Of 21 strains from one culture of *B. tabacum* 21 were alive and had not changed after 597 days, but after 960 days only 13 were alive, of which 8 produced both wildfire and angular leafspot. After 1,456 days only one culture was viable and it produced only wildfire. Of 44 strains from the other culture of *B. tabacum* 41 produced wildfire and 1 either virulent angular leafspot or very weak wildfire after 307 days. After

654 days one strain produced both wildfire and angular leafspot and 33 only wildfire. After 1,157 days 1 culture produced only angular leafspot, 34 produced only wildfire, while 6 produced both wildfire and angular leafspot.

These tests furnish further proof of the close relationship of these two groups of organisms and suggest that change can take place in either direction. Observations in nature suggest that *B. angulatum* is the organism from which *B. tabacum* arises by mutation.

Seed Contamination

Tobacco seed has been considered by some investigators to be the chief source of infection of tobacco with the leaf-spotting bacteria. No adequate proof of this theory has been published; and seed treatment, together with other sanitary measures, has failed to control the diseases in the plant bed. The following studies were designed to determine the relation of seed contamination to plant-bed infection.

Inoculated seed.—A small bed was sowed March 27, 1933, with seed soaked 5 hours in a broth culture of *B. angulatum* and then dried. On May 11 a few suspicious spots were found which, by inoculation tests, were proved to contain *B. angulatum*. The disease did not develop beyond this stage.

Seed soaked in a broth culture of *B. angulatum* for 3 hours was dried and sowed in a flat of steamed soil in the greenhouse. Shortly after germination, 1.6 percent of the seedlings had a yellowish-brown spot on the tip of one cotyledon. Other seedlings with the seed coat still clinging to the tip of one cotyledon were observed. Ten of the discolored cotyledons were dropped into individual tubes of broth which were used as inoculum 24 hours later. In each case typical angular leafspot developed. Apparently, in a few instances (1.6 percent), *B. angulatum* had spread from the contaminated seed coat to the tip of one cotyledon.

The cotyledons of untreated seed grown without bagging in the field are remarkably free from any abnormalities. In 1933 an extensive bed sowed with untreated seed was examined for infected cotyledons. Three were found which appeared suspicious, but inoculations from them proved to be negative. Angular leafspot subsequently developed suddenly and extensively in this bed.

Natural seed contamination.—As plant-bed tests for seed contamination proved unsatisfactory, a method of testing seed, dried bits of trash, and leafspots was used in the following studies. It proved successful where known contaminated material was used

(Table 5, page 21). Seeds or bits of material to be tested were put into broth and kept 24 to 48 hours. Then the broth was atomized on the lower surface of leaves of greenhouse plants. The development of typical leafspots was considered proof of the presence of the pathogenic organism in the material tested. From 20 lots of 1932 seed sent to the Experiment Station for cleaning, a sample each of trash, trash and seed, and cleaned seed of 14 lots was tested in duplicate during February and March, 1933. In 84 tests no angular leafspot developed. The other 6 sets of samples were tested once, but with negative results. Thirty more samples were saved in the seed laboratory in 1933 and tested in the same way in triplicate. A total of 180 inoculations were negative.

Seed was tested from a field on the Experiment Station farm in 1933 where angular leafspot had spread extensively. The pods were crushed, sieved and run through a cleaner which left small bits of pods and considerable dust with the seed. Of eighty-three $\frac{1}{4}$ -gram samples (each sufficient to sow 25 square feet of bed, or a total of 230 feet of bed 9 feet wide) of this seed, of which 39 had been recleaned to remove dust, one sample each of the cleaned and recleaned seed caused angular leafspot.

Two seed heads with natural infections (from which *B. angulatum* was isolated) were found in the summer of 1933. Seed poured from 7 ripe pods into tubes of broth proved to be sterile 9 days later, as the broth was clear. Bits of calyx lobes from 3 of the 7 pods, and capsule walls from 2 of the 7 caused angular leafspot.

Seed-pod inoculations in the field.—In 1932 two inflorescences of burley tobacco in the field were sprayed several times at intervals

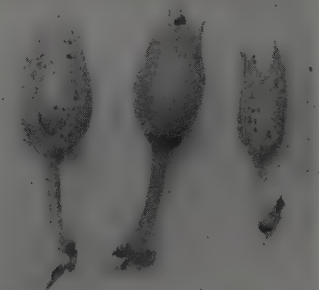


Fig. 3.—Angular leafspot on pods sprayed 8 times in 22 days with *B. angulatum*.

with pure cultures of *B. angulatum* and kept covered with a paper bag. About 90 days after harvest, bits of capsule walls in 3 tubes and 2 of 4 tubes containing bits of calyx lobes caused typical angular leafspot. One of 13 small masses of seed containing bits of trash, caused angular leafspot. Ten small masses of seed from which all bits of trash were removed, proved negative. Three of 5 tests of seed and small bits of trash gave typical angular leafspot.

Inflorescences were sprayed as many as 11 times over a period of 15 days with pure cultures of *B. angulatum* and *B. tabacum* at all stages of development from the early bud to ripened open pods. Pod and calyx spots which developed were small, dark and usually not numerous (Fig. 3). Seed from 13 of a total of 47 pods, from seed stalks sprayed after pods had opened, were found to be contaminated with *B. angulatum*. When inoculation ceased before capsules split, seed infection was rare (Table 4).

TABLE 4.—EXTENT OF SEED CONTAMINATION WITH *B. angulatum*
WHEN INOCULATION CEASED BEFORE CAPSULES OPENED

Times capsules were inoculated	Number of capsules tested	Seed positive for <i>B. angulatum</i>
3	12	0
4	5	0
6	9	0
9	23	0
1	12	0
13	18	0
1	15	0
16	24	1
17	14	0
Total	132	1

During the summer of 1933 flower heads in the field were inoculated and then covered with paper bags. Four heads were inoculated with *B. tabacum* and 9 with *B. angulatum* at intervals until the first capsules were nearly ripe. The capsules were allowed to ripen in the field and were then hung in the laboratory. Seed, bits of calyx lobes, and capsule walls were tested during November. Seventy-nine percent of the bits of capsule wall and calyx tested were found to be contaminated with one or the other organism (Table 5). Seed from 4 of 73 capsules inoculated with *B. tabacum* and 4 of 158 capsules inoculated with *B. angulatum* were found contaminated with the respective organisms. Considering the number of times and the dif-

TABLE 5.—EXTENT OF SEED, CAPSULE, AND CALYX CONTAMINATION WITH *B. tabacum* AND *B. angulatum* IN FIELD-GROWN, INOCULATED PODS

Plant No.	Number of times inoculated	Total tests of					
		Seed		Capsule wall		Calyx	
<i>B. tabacum</i>							
		No.	Positive	No.	Positive	No.	Positive
15	5	22	0	—	—	15 ^a	12
75	10	17	0	9	7	9	8
83	10	17	1	9	8	9	6
36	11	17	3	9	8	9	7
Totals		73	4	27	23	42	33
<i>B. angulatum</i>							
18	4	22	0	—	—	16 ^a	10
93	9	17	2	9	9	9	6
87	9	17	0	9	5	9	8
53	10	17	1	9	8	9	7
49	11	17	0	8	7	8	8
46	11	17	1	9	7	9	6
47	11	17	0	9	9	9	7
55	9	17	0	9	7	9	6
99	9	17	0	9	6	9	7
Totals		158	4	71	58	87	65

^a Capsule walls and calyx lobes tested together.

ferent stages at which inoculations were made, the large amount of inoculum applied and the possibility that small bits of contaminated capsules may have fallen into the tubes, the small number of seed contaminations may be almost disregarded. It appears probable that the seeds within the capsule do not become contaminated but contamination occurs only when bits of infected trash are mixed with the seeds.

Seed-pod inoculations in the greenhouse.—A series of inoculations were made in the greenhouse at all stages of blooming and pod formation, with the atomizer held rather close to the flower parts. The pods were allowed to ripen on the plants, then removed, the tip clipped off and the seed poured into a tube of broth. Of a total of 132 pods tested, many of which had been inoculated more than 10 times, seed from but one capsule appeared to be contaminated with *B. angulatum* and only 30 percent of the tubes were contaminated with any organism.

From studies on natural and artificial infection of tobacco seed heads it may be concluded that tobacco seed rarely if ever becomes infected with either organism, but may become contaminated if the seed pods are open when inoculum is dispersed, and that pods and calyx lobes may be infected but that even this is comparatively rare. Seed contamination does not therefore appear to be an adequate explanation of plant-bed infection.

Two-year-old seed.—To investigate the likelihood that *B. angularatum* would live in trash through the second winter, bits of calyx lobes and seed pods of inflorescences inoculated during the summer of 1932 were tested December 20, 1933, 459 days after harvest. No angular leafspot developed in 20 tests each of calyx lobe and seed-pod material. Inoculations with 10 angular leafspots and 10 wildfire spots, produced by pure culture inoculations, from leaves dried in the laboratory 421 days, were negative. This would indicate that 2-year-old seed would be entirely safe for sowing if seed contamination were common. These tests confirm studies made by others on the longevity of *B. tabacum* in dried tissue.

Thermal death time of *B. angularatum* at 52° C.—The thermal death point of *B. angularatum*, as reported by Wolf and Foster (49) is 45° to 51° C, and of *B. tabacum*, 46° to 51° C for 10 minutes. Stapp (41) found it to lie between 47° and 49° C for both organisms.

In culture.—In the present studies, in determining length of time required to kill *B. angularatum* at 52° C 48-hour broth cultures were used. Three drops of broth were transferred to 8 cc test tubes containing 3½ cc of this broth heated to 52° C in a water bath. The tubes were then quickly replaced in the water bath where they were maintained for the periods indicated in Table 6 at a temperature of 52° C. Bacteria were usually killed at an exposure of 8 minutes at 52° C, but in some instances exposures of 9, 10, and 11 minutes failed to destroy all bacteria. In one tube exposed for 11 minutes cloudiness did not develop until 5 days after treatment. In others it developed in 3 days.

In cured leaves.—As leafspot bacteria appear to be carried only in bits of naturally infected calyx lobes or capsule tissue and not in seed, a determination was made of the thermal death time of the

TABLE 6.—EFFECT OF WATER TREATMENT FOR DIFFERENT PERIODS OF TIME
AT 52° C. ON *B. angularatum* IN CULTURES (6 TRIALS)

Minutes treated	1st trial (duplicated)	2nd trial (duplicated)	3rd trial	4th trial (duplicated)	5th trial (duplicated)	6th trial (duplicated)
11	— ¹ —	— —	—	— + ²	— —	— —
10	— —	+ ³ +	—	+ +.	— —	— —
9	— —	+ +	—	— —	— —	— —
8	— —	— —	—	— +	— —	— —
7	+ +.	+ —	—	+ +.	— —	+ +.
6	+ +	+ +	—	+ +.	+ +.	+ +.
5	+ +	+ +	+	+ +.	+ +.	+ +.

¹ — = culture medium remained clear following treatment.

² +. = clouded medium produced angular leafspot.

³ + = culture medium cloudy following treatment.

TABLE 7.—EFFECT OF WATER TREATMENT AT 52° C. ON *B. angulatum* AND *B. tabacum* IN DRIED LEAFSPOTS

Number of days tissue dried	Minutes of treatment	No. tissues tested	No. of inoculations positive	Controls not treated	
				Number tested	Number positive
<i>B. angulatum</i>					
129	20	20	0	20	5
10	20	20	0	20	19
11	18	20	0	10	10
15	15	20	1	20	20
13	13	20	0	10	8
16	10	20	0	10	9
14	8	30	0	15	12
19	6	30	0	15	15
17	4	30	23	15	15
23	3	30	26	15	15
Total				150	128
<i>B. tabacum</i>					
122	20	20	0	20	18
21	18	20	0	10	10
11	15	20	0	10	10
13	13	20	0	10	10
16	10	20	0	10	8
14	8	30	0	15	9
19	6	28	0	14	7
17	4	30	14	15	15
23	3	30	28	15	14
Total				119	101

organism in dry leaf tissue. Spots were cut from artificially inoculated leaves in the field or greenhouse, and dried. After drying for varying lengths of time tests were made to determine the time required to kill the organisms when placed in water at 52° C. The bits of tissue were placed in a damp chamber until moist, then placed in a small piece of cheesecloth and soaked in water for about one hour. The cheesecloth bag and bits of tissue were then treated in water at 52° C for periods varying from 3 to 20 minutes. Then 3 or 4 of the treated bits were placed in each of 20 or 30 test tubes of broth. Control tissue soaked in water an equal time were transferred to other tubes. After about 24 hours, inoculations were made to burley tobacco plants in the greenhouse with an atomizer. The results are given in Table 7.

With one exception all tissue exposed 6 minutes or longer failed to give infection, while more than 80 percent of the controls of both *B. angulatum* and *B. tabacum* were positive. Three-minute exposure appeared to have no effect on either organism. These tests would seem to prove that a hot water seed treatment of 20 minutes at 52° C, especially if preceded by a presoak of 1 hour, should be more than adequate to completely destroy *B. angulatum* or *B. tabacum* on the seed or in bits of tissue mixed with the seed.

Hot-water seed treatment.— Oros (38) concluded in 1932 that hot-water treatment of tobacco seed was of no value against fungi and bacteria present on the seeds. Seed treated 2 minutes in water at 65° C germinated normally, while seed treated for 2 minutes at 73° C did not germinate. In the present studies longer exposures at lower temperatures were used.

A hot-water seed treatment for tobacco seed, developed about 1926, consisted in soaking seed in water at 52° C for 20 minutes. This treatment was used on hundreds of small lots of seeds during several years in out-of-door plant beds with satisfactory germination and vigor of plants.

The percentage germination of tobacco seed treated in water at various temperatures and for various exposures in order to determine the limits of heat treatment is given in Table 8. Following treatment, the seeds were germinated on moist blotters at 26° C in reduced light, with or without drying. The undried seeds germinated poorly. Germination of dried seed was much better. Germination did not appear to be appreciably affected except at and above 52° C 1 hour, 54° C 45 minutes, 56° C 25 minutes, and at 57° C 20 minutes. Germination was normal following exposure at 54° C for 30 minutes and 52° C for 45 minutes and much longer periods at lower temperatures. All hot-water treatments delayed germination slightly as compared with seed soaked in water at room temperature, but germination was decidedly delayed but otherwise normal, following treatments at 52° C, 30 minutes; 54° C, 20 minutes; 56° C, 15 minutes, and 57° C for 12 minutes. Three hours treatment at 48° C and 2 hours at 50° C had no injurious effect on germination.

In these trials, treatment at 52° C for 20 minutes and at 54° C for 15 minutes appeared to be safe. Therefore a test of seed of 10 burley varieties was made at these temperatures and exposures. Treatment for 20 minutes at 52° C seemed to improve germination. Treatment at 54° C for 15 minutes reduced germination and delayed it slightly. After 8 days drying, 7 of the 10 lots treated at 54° showed that a portion of the seedlings were somewhat stunted.

In another test seed of the same 10 varieties was presoaked one hour in water at 26° C, treated for 20 minutes at 52° C, dried 2 days, and 200 seeds of each variety sowed in soil which had been heated

¹The work on hot-water seed treatment was done prior to the tests which showed that seeds rarely, if ever, carry the pathogens. It is not believed that it will be of any value in the control of the leafspot diseases but is published at this time in the hope that it may be of some value if a hot-water treatment is desired for the control of some other disease of tobacco.

TABLE 8.—GERMINATION PERCENTAGE OF TOBACCO SEEDS AFTER TREATMENT WITH WATER AT DIFFERENT TEMPERATURES FOR DIFFERENT LENGTHS OF TIME

(Germination tests were started immediately after treatment or after the seeds had been dried at room temperature for 24 hours or for 4 weeks.)

When germination test was started	Treatment time (mins.)	57° C (135° F)	56° C (133° F)	54° C (129° F)	52° C (126° F)	50° C (122° F)	48° C (118° F)	46° C (115° F)	Room temperature
Immediately	3	33	—	—	—	—	—	—	—
	5	—	16	48	—	—	—	—	—
	6	37	—	—	—	—	—	—	—
	9	16	—	—	—	—	—	—	—
	10	—	17	19	—	—	—	—	—
	12	15	—	—	—	—	—	—	—
	15	15	20	44	29	—	—	—	—
	20	—	8	24	67	34	—	—	—
	25	—	7	4	13	40	—	—	—
	30	—	—	—	17	53	—	—	86
	35	—	—	—	—	42	—	—	90
	45	—	—	—	—	—	21	—	—
	60	—	—	—	—	—	12	37	55
	90	—	—	—	—	—	19	33	59
	120	—	—	—	—	—	21	37	—
	150	—	—	—	—	—	—	33	—
	180	—	—	—	—	—	—	16	54
Average		23	14	27	33	42	18	31	60
After 24 hours	10	92	—	—	—	—	—	—	—
	15	92	—	—	—	—	—	—	—
	20	83	94	—	—	—	—	—	—
	25	77	84 ^a	—	—	—	—	—	—
	30	—	47 ^a	96	95	95	—	—	—
	35	—	71 ^a	—	—	—	—	—	—
	40	—	38 ^a	—	—	—	—	—	—
	45	—	—	77 ^a	92	95	90	—	—
	60	—	—	70 ^a	82	93	90	—	—
	75	—	—	19	91	99	—	—	—
	90	—	—	25 ^a	74 ^a	92	92	—	—
	105	—	—	—	—	96	—	—	—
	120	—	—	—	—	92	98	—	—
	150	—	—	—	—	—	94	—	—
	180	—	—	—	—	—	95	—	—
Average		86	67	57	87	95	93	—	90
After 4 weeks	3	97	—	—	—	—	—	—	—
	5	—	85	92	—	—	—	—	—
	6	87	—	—	—	—	—	—	—
	9	86	—	—	—	—	—	—	—
	10	—	90	92	—	—	—	—	—
	12	72	—	—	—	—	—	—	—
	15	50	81	93	91	—	—	—	—
	20	—	66	87	87	68	—	—	—
	25	—	40	86	88	66	—	—	—
	30	—	—	94	(87 92)	(97 96)	—	—	95
	35	—	—	—	—	93	—	—	—
	45	—	—	—	90	96	72	—	—
	60	—	—	—	80	90	87	92	95
	75	—	—	—	91	91	—	—	—
	90	—	—	—	81	96	—	87	85
	105	—	—	—	—	96	—	—	—
	120	—	—	—	—	83	76	83	—
	150	—	—	—	—	—	—	81	—
	180	—	—	—	—	—	—	77	90
Average		78	72	91	87	88	75	84	91
Not treated									95

^a Some weak plants but majority strong.

to 65° C for one hour. The checks were untreated. A second seeding was made 24 days later. The presoaked and treated seed of the majority of the varieties germinated better in both tests. The seedlings from treated seed of two varieties kept 24 days, were noticeably smaller. It may be concluded that for immediate sowing, tobacco seed may be presoaked an hour and treated 20 minutes at 52° C without injury to germination and in the majority of cases may be saved 24 days without injury.

HOST RANGE OF *B. ANGULATUM* AND *B. TABACUM*

J. Johnson *et al* (33) showed by inoculation experiments that typical wildfire spots could be caused on many species of plants with cultures of *B. tabacum*. Clayton (13) then proved that sterilized culture medium in which *B. tabacum* had been grown could likewise cause typical wildfire spots on many species. He believed that the toxin contained in the medium was the cause of the spots in Johnson's inoculations and concluded that the only hosts of *B. tabacum* were species of *Nicotiana*. However, Wolf and Foster (50) had already shown that cowpeas were susceptible and Chapman and Anderson (11) proved that tomato, petunia and pokeweed were susceptible.

Beach (5) reported that *Physalis virginiana* growing near wildfire-affected tobacco plants frequently developed typical wildfire spots from which *B. tabacum* could be isolated. Valleau and Johnson showed in 1935 (44) that *Physalis subglabrata* M & B sometimes is a natural host of *B. angulatum*. In a study of colony characters of *B. angulatum* obtained from *physalis*, one colony was selected which caused typical wildfire on burley and dark tobacco in repeated tests, and others from the same plate which caused typical angular leafspot. Moore (36) found wildfire on *Nicandra physaloides*.

In 1938 and frequently since that time morning-glory (*Ipomea hederacea* Jacq.), growing as far away as 250 feet from wildfire-infected tobacco beds, has been found infected with wildfire. Inoculations from the spotted leaves to tobacco have given typical wildfire, and inoculations of morning-glory with pure cultures of *B. angulatum* and *B. tabacum* have produced typical angular leafspot and wildfire respectively (Fig. 4).

In 1941 Allington (1) reported that following water-soaking many species of plants might be infected with *B. tabacum* and *B. angulatum* and that these organisms may survive as long as 6 months in the dried tissues. He suggested that this dried material might act as a source of field infection.

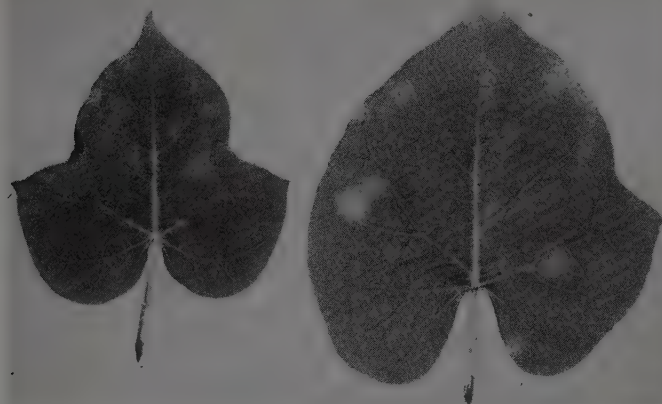


Fig. 4.—*Ipomea hederacea* Jacq. leaves 11 days after inoculation with *B. angulatum* (left) and *B. tabacum* (right). Printed direct from the leaf to direct positive paper by transmitted light.

In 1936, using pure cultures of *B. tabacum* and *B. angulatum*, plants of 4 varieties of cowpeas (*Vigna sinensis*), one variety of soybean (*Soja max*), 2 varieties of tomatoes (*Lycopersicum esculentum*) June Pink and Marglobe, 2 varieties of pepper (*Capsicum annuum*) Ruby King and Red Chili, *Datura stramonium* and *Nicotiana glutinosa*, were inoculated on tender leaves with an atomizer. When infection was evident, bits of tissue from each were examined under the microscope. In each there was evidence of abundant multiplication of the organisms. Other bits of infected tissue from these plants were then crushed in sterile water and inoculations made to another specimen of the same species. In this way the two organisms were carried through 10 successive plants of each species in each of which typical infection and multiplication occurred. Under greenhouse conditions each species appeared to be as good a host as tobacco.

A similar series was attempted on *Nicotiana rustica*, *N. sanderae*, *N. Langsdorffii*, *Physalis subglabrata* and *Solanum carolinense*. In these species infection and multiplication occurred in some specimens but serial transfers through 10 successive plants were not accomplished. It is of interest to note that whereas both organisms are found in nature in old flea-beetle-gnawed leaves of *Physalis subglabrata*, greenhouse-grown plants of this species usually proved highly resistant to pure-culture inoculations although occasionally infection with mul-

tiplication of bacteria occurred. Tomatoes grown in tobacco beds have been found infected with *B. angulatum* and *B. tabacum*.

With the knowledge (evidence to be presented later in this bulletin) that the bacteria multiply on the roots of crop plants and weeds it seemed desirable to determine at least some of the plants on which they grow. Crop plants which have been found to be susceptible to root infection are tobacco, wheat, rye, barley, hairy vetch, lespedeza, red clover, crimson clover, white clover, and orchard grass; Italian rye grass and Kentucky bluegrass appear to be only slightly susceptible. Infection was obtained from the roots of the following weeds: ragweed, quack grass, shepherd's purse, giant ragweed, chickweed, vermifuge, and oxalis. No attempt has been made to determine the complete host range but the results given indicate that it is extensive and that root-susceptible plants may be expected to be present nearly any place selected for a plant bed.

INFECTION STUDIES

Sources of Infection

Examination of burley plant beds in Fayette and neighboring counties showed that angular leafspot was visibly present in about 90 percent of the beds each year when control measures were not used. In dark tobacco beds the disease was not so evident but it was present in the majority of fields.

Seed.—In Virginia it was concluded from plant-bed tests that about 25 percent of the seed samples were contaminated with *B. angulatum*, and that a seed treatment prevented plant-bed infection if other sanitary precautions were followed (23). In the present studies we have been unable to demonstrate that seed contamination plays any appreciable part in plant-bed infection. Hot-water-treated seed, chemically-treated seed, seed raised in the greenhouse, and old seed all failed to give control in the plant bed when all other known precautions were taken to prevent infection. Further, we have shown that seed contamination is rare. It is therefore evident that there are sources of plant-bed infection other than seed and materials used in preparing the bed.

Windblown trash.—Various workers (3, 4, 5, 32) have suggested that wind-blown contaminated trash might play a part in plant-bed infection with wildfire. If infection occurred in this way, the disease would begin in isolated spots in the bed and its progress from these spots could be followed. While this occurs in some instances, es-

pecially with wildfire, angular leafspot commonly appears suddenly over much of the bed with little evidence of gradual spread or of previous infection. If windblown trash were an important factor, it would be expected that beds on the Station farm would be heavily infected with wildfire each year as they lie east of a very large loose leaf market from which much tobacco trash must be blown. However, wildfire has been found in appreciable amounts only twice in the past 23 years in the Station beds.

Soil.—Beach (6) reported that *B. tabacum* overwinters in the soil and that the disease frequently is controlled with no other precaution than the use of new plant-bed sites. Wsorrow (51), studying a similar disease of tobacco which he attributed to a new organism, *Phytomonas heterocea*, believed that the chief sources of infection were seed and soil and that soil infection was general. Johnson and Murwin (32) state that repeated experiments indicate that wildfire bacteria cannot survive more than a month in moist soil. Anderson and Chapman (4) collected evidence to show that healthy plants set in the field might become infected before the season was over. Clinton and McCormick (16) collected soil in March and April from a garden plot where wildfire-affected plants had grown the year before and had been allowed to rot in place. Infection was obtained on three plants from this soil soaked in water several hours.

Because of numerous observations of sudden and general outbreaks of angular leafspot in plant beds following a few hours' rain, the conclusion was reached by the writers several years ago that the inoculum probably originated from some source unrelated to a previous tobacco crop. Consequently a study was made of leafspot diseases of other crops and wild plants growing in the vicinity of tobacco beds. Several were found to be affected by bacterial diseases but none was found which could possibly be the source of plant-bed infection. Another year insects were collected in large numbers from tobacco beds and their vicinity. These were crushed in broth and the broth used as inoculum. Only negative results were obtained, indicating that insects were probably not the means by which the bacteria were brought into the bed. It then appeared that the leafspot organism must be present in the plant-bed soil. Therefore a number of samples of soil were collected from plant beds while the plants were small and before leafspot had developed. Only one sample of a large number tested was found to contain the angular leafspot organism and this was from a bed site which had been used the preceding year.

Nevertheless, studies on overwintering of the organisms in the

TABLE 9.—EXTENT TO WHICH *B. angulatum* AND *B. tabacum* OVERWINTERED IN FIELD PLOTS ON WHICH TOBACCO INFECTED WITH ANGULAR LEAFSPOT AND WILDFIRE WAS GROWN IN 1940

Dates collected	Number samples	Number causing infection	Total angular leafspots
Rye cover crop. (Angular leafspot had been present)			
11-2-40 to 1-8-41	50	26	1395
2-4-41 to 2-25-41	48	2	13
3-3-41 to 3-19-41	48	1	40
4-7-41 to 4-17-41	48	8	17
5-8-41 to 7-16-41	72	0	0
Fusarium plot (Wildfire and angular leafspot both had been present.)			
		Wildfire spots	
8-27-41 to 10-20-41	50	23	258
11-13-41 to 1-13-42	50	20	361
1-22-42 to 3-17-42	50	11	28
3-24-42 to 4-21-42	30	2	3
5-12-42 to 5-22-42	20	0	0
Burley breeding plot (Angular leafspot had been present.)			
8-27-41 to 10-20-41	50	15	1722
11-13-41 to 1-13-42	50	18	253
1-22-42 to 3-17-42	50	12	48
3-24-42 to 4-21-42	30	6	74
5-12-42 to 5-22-42	20	0	0

soil were begun in the fall of 1940. Six small plots of soil out-of-doors, previously steamed, were artificially inoculated with *B. angulatum* and 3 areas in tobacco fields naturally infected with angular leafspot (one contained a little wildfire) were charted and used for study. To determine whether or not pathogenic bacteria were present in the soil, enough soil to fill a tumbler about one-third full was collected, water was added and the mass stirred and poured over the under surface of a tobacco leaf previously water-soaked.¹ Under these conditions pathogenic bacteria, if present, are likely to pass through the stomata (together with much other material in solution and suspension) and cause infection. Water-soaking quickly disappears, and the typical spots can be seen after a few days. For a time microscopic examinations of the spots and reinoculations from spots to healthy leaves were made to further prove the pathogenicity of the organisms.

It was found that the typical spots produced directly from soil inoculations contained a mass of motile bacteria which had undoubtedly multiplied in the leaf. These were capable of causing infection when inoculated into other leaves. Both from artificially inoculated

¹ Water soaking was accomplished with a 20 cc injecting syringe, the needle of which had been cut off square. The syringe was filled with water and the stream directed against the underside of the leaf. During the day when light conditions are right water soaking occurs immediately.

TABLE 10.—NUMBER OF ANGULAR LEAFSPOTS PRODUCED BY MIXING A COMPOSITE OF 10 SMALL SOIL SAMPLES WITH WATER AND POURING IT ON A WATER-SOAKED TOBACCO LEAF

(The plots were inoculated October 11, 1941, with 2 pure cultures of the organisms or as indicated)

When tested	Plots with cover crop			Fallow plots		
	Crimson clover	Vetch	Wheat	2 pure cultures	Mixed cultures	Inoculated dried tobacco leaves
1941						
Oct. 22	600	400	600	400	400	300
Nov. 14	6	19	67	2	23	0
Dec. 6	17	116	50	1	1	500
Dec. 15	7	15	39	300	0	0
Dec. 23	45	6	142	2	0	2
1942						
Jan. 13	14	69	5	0	0	0
Jan. 22	31	69	16	0	0	0
Feb. 5	66	6	500	1	0	11
Feb. 12	80	180	180	16	9	0
Feb. 19	108	54	60	3	5	53
Mar. 4	31	18	74	0	5	0
Mar. 17	50	72	12	0	0	0
Apr. 2	30	14	11	0	0	0
Apr. 20	33	320	23	0	3	0
May 5	11	9	4	0	0	0
May 23	0	0	0	0	0	0

soil and from field soil naturally contaminated, *B. angulatum* and *B. tabacum* were isolated many times in tobacco leaves during two winters (20), proving conclusively that the organisms are capable of maintaining themselves in soil for at least 6 months, a sufficient time to tide over between one tobacco crop and the next (Table 9). Incidentally a method was made available to the plant pathologist for isolating from the soil any bacteria which may be pathogenic on leaves of plants.

Cover crop roots.—In the fall of 1941, 3 of the artificially contaminated plots mentioned on page 30 were sowed with wheat, vetch, and crimson clover, respectively, and 3 were left fallow. In the latter, a few winter weeds developed. There was evidence throughout the winter of more consistent results in causing infection from the cover-crop plots than from the fallow plots (Table 10). This, combined with negative results from tests of plant-bed soils in the spring of 1940, suggested that the bacteria might be living on the roots of cover crops and not in the soil. Therefore, roots of the cover crops were dug, carefully washed in running water to remove the dirt, ground in a mortar, water added, and the decoction poured over the under surface of water-soaked tobacco leaves.¹ Infection, sometimes very

¹ The first observable effect of inoculation with crushed washed roots of plants on tobacco leaves was the appearance, about the second day, of chlorotic streaks

(Footnote concluded on following page)

TABLE 11.—INFECTION PRODUCED BY FIELD SOIL, SOIL TAKEN FROM THE ROOTS, AND BY THE WASHED ROOTS OF COVER CROPS, FROM FARMERS' FIELDS IN WHICH LEAFSPOT DISEASES WERE PREVALENT IN 1941

(Each sample of soil or roots was a composite of 10 collections)

	Field K. Rye cover crop. Wildfire in 1941 tobacco; no angular leafspot seen				Field R. Wheat cover crops ^a	Field W. Barley cover crop. Angular leafspot in the 1941 tobacco		
When sampled	3-30-42	4-15-42	5-5-42	6-1-42	4-13-42	4-2-42	4-27-42 ^a	5-4-42
Number of soil samples	8	10	10	—	10	10	10	10
Number causing infection	2	1	0	—	2	6	0	0
Average number of spots	1 ^b	1	—	—	28	8	—	—
Number of soil samples shaken from roots	8	—	—	—	10	10	—	—
Number causing infection	8	—	—	—	9	3	—	—
Average number of spots	7 ^b	—	—	—	84 ^c	1	—	—
Number of root samples	8	50 ^d	50 ^d	10	10	10	50 ^d	48 ^d
Number causing infection	8	13 ^e	5	1	19 ^f	8	3	11
Average number of spots	27 ^b	27	3	3	135 ^g	5 ^h	68 ^h	2 ^h

^a Both wildfire and angular leafspot in the 1941 tobacco. ^b Wildfire. ^c 47 wildfire and 37 angular leafspot. ^d Single plants. ^e 12 wildfire and 1 angular leafspot. ^f 9 wildfire and 10 angular leafspot. ^g 72 wildfire and 63 angular leafspot. ^h Angular leafspot.

heavy, usually followed inoculation with roots of each of the cover crops and from some of the weeds in the fallow plots. Roots of wheat, barley, and rye were then collected from fields in which tobacco was heavily infected the previous summer with wildfire, angular leafspot, or both. Three tests were made from some fields, using soil, the soil shaken from the roots, and the washed roots. It was demonstrated that the ground washed roots caused much heavier infection than the soil, or soil shaken from roots before washing. The results shown in Table 11 indicate that both organisms are capable of passing the winter in fields in which an infected tobacco crop was grown the previous summer, if a cover crop is present (46).

(Footnote continued from page 31)

which exactly corresponded with the water-soaked areas. Apparently some toxic agent capable of rapidly breaking down chlorophyll is present in crushed root tissue. Possibly because of this toxic material, pathogenic organisms sometimes did not develop sufficiently to make an observable spot. Their presence was detected in some instances by microscopic observation, and by using bits of yellow tissue as inoculum on healthy leaves where sometimes angular leafspot, wildfire, or both developed. This fact is mentioned to show that it is not safe to consider that the pathogenic organisms are not present on roots because of negative evidence in the first inoculation on leaves. However, in the present study, development of typical spots on first inoculation was required for proof of pathogenic organisms on the roots.

Tobacco roots in the plant bed.—Tobacco plants were then collected at random from plant beds in Fayette and neighboring counties, the roots washed and inoculations made. While the plants were small and the roots presumably in the zone of soil which had been partially sterilized, either by burning or steaming, no pathogenic bacteria appeared to be on the roots (Table 12). The season was dry until after the first week in May. By May 11 angular leafspot and wildfire were developing in many beds and from that time on collections of washed roots from beds which had not been treated with Bordeaux mixture frequently gave heavy infection, and roots from 4 beds of a total of 16 treated with Bordeaux produced infection. Eight samples which produced positive results were from beds in which the disease was not yet evident on the leaves. These results strongly suggest that the bacteria first develop on the roots of the plants in the bed and later are carried to the leaves during a period of wet weather when the soil is wet and the leaves water soaked.

In the spring of 1943, at setting time, further collections of tobacco roots were made from treated and untreated beds. Plants were collected from 20 different places in each bed. Twenty collections from 20 bordeaux-treated beds resulted in 19 negative and 1 which caused angular leafspot. Collections from 5 untreated beds in which disease had already appeared gave 3 with both wildfire and angular leafspot, 1 with wildfire, and 1 negative. The angular leafspot and wildfire spots produced by these inoculations were in every way identical with spots as they occur on tobacco naturally or spots produced on similar plants with pure cultures of the organisms isolated from typical leafspots. The results of these tests while not conclusive

TABLE 12.—INOCULATIONS FROM ROOTS OF TOBACCO PLANTS FROM PLANT BEDS, 1942

Date of collection	Number of beds		Number of beds with		Result of inoculation			
	Not treated	Bordeaux treated	wildfire	angular leafspot	Number of			
					wildfire spots		angular leafspots	
					Range	Average	Range	Average
4-28 to 5-1	7	21	0	0	0	0	0	0
4-29	1	0	0	0	0	0	0	400
5-11 to 6-2	—	12	0	0	0	0	0	0
5-11 to 6-2	—	4	0	0	0	0	2-69	22
5-11 to 6-2	12	0	0	12	0	0	2-2000	342
5-11 to 6-2	12	0	0	0	0	0	0	0
5-11 to 6-2	3	0	0	0	0	0	3-200	96
5-11 to 6-2	2	0	0	2	0	0	0	0
5-11 to 6-2	2	0	1	0	0	0	0	0
5-11 to 6-2	1	0	1	0	0	0	0	0
5-11 to 6-2	4	0	4	0	100-500	200	0	0
5-11 to 6-2	2	0	2	0	0	0	30-400	50

TABLE 13.—PATHOGENICITY OF BACTERIAL COLONIES FOUND ON ROOTS OF TOBACCO PLANTS AFFECTED BY WILDFIRE AND ANGULAR LEAFSPOT IN THE PLANT BED

(A bit of root bearing the colony was used as inoculum on water-soaked tobacco leaves)

	Inoculum from roots of plants affected by	
	Wildfire	Angular leafspot
Number tested	59	53
Number producing leafspots	50	46
Average	57	32
Number of spots produced	1 to 300	2 to 200

suggest that the bluestone lime treatment tends to lessen the chance that roots of tobacco plants in treated beds will become infected.

Washed roots of tobacco plants growing in naturally infected beds were next examined to determine whether the colonies of bacteria could actually be seen on the roots. Lens-shaped colonies of bacteria apparently embedded in a gelatinous matrix were frequently found. If watched for some time, in water, motile bacteria could be seen escaping from the surface of the mass of some colonies while the remaining bacteria were entirely without motion. Other colonies seemed to be very tenacious and could be left in water for hours without apparent escape of bacteria. Bits of roots bearing these colonies, when removed, crushed in water, and used as inoculum, frequently caused angular leafspot or wildfire, depending upon the source of the material (Table 13).

Tobacco roots in the field.—Tobacco roots, collected within 5 weeks after setting, from fields set with plants from beds infected with wildfire or angular leafspot have frequently given heavy infection on water-soaked leaves. This and the fact that the bacteria are found on roots of plants in the bed before leaf spots develop suggest that the bacteria are carried from the bed to the field not only in leafspots but also on tobacco roots. Throughout the summer and

TABLE 14.—LEAFSPOT BACTERIA FROM TOBACCO ROOTS COLLECTED FROM TOBACCO FIELDS ON 12 FARMS BETWEEN JUNE 24 AND AUGUST 28, 1942

(A collection consisted of from 1 to 20 roots, depending upon the size of the roots)

Disease on leaves	Number of collections	Number of collections producing	
		Wildfire	Angular leafspot
None	17	0	14
Wildfire	19	19	2
Angular leafspot	27	3	17

until after harvest and time for sowing cover crops, wildfire and angular leafspot organisms have also been isolated repeatedly from tobacco roots in fields infested with one or the other of these organisms (Table 14). Frequently typical angular leaf spots or wildfire spots numbering in the hundreds have developed on the inoculated leaves, leaving no doubt whatever of the presence of these organisms on tobacco roots throughout the summer.

Grass roots.—Roots of wheat, grown from surface-sterilized seed in a damp chamber produced numerous colonies when inoculated (Fig. 5), but roots of bluegrass and Italian ryegrass appeared to be a poor medium except that occasional seedlings have colonies.

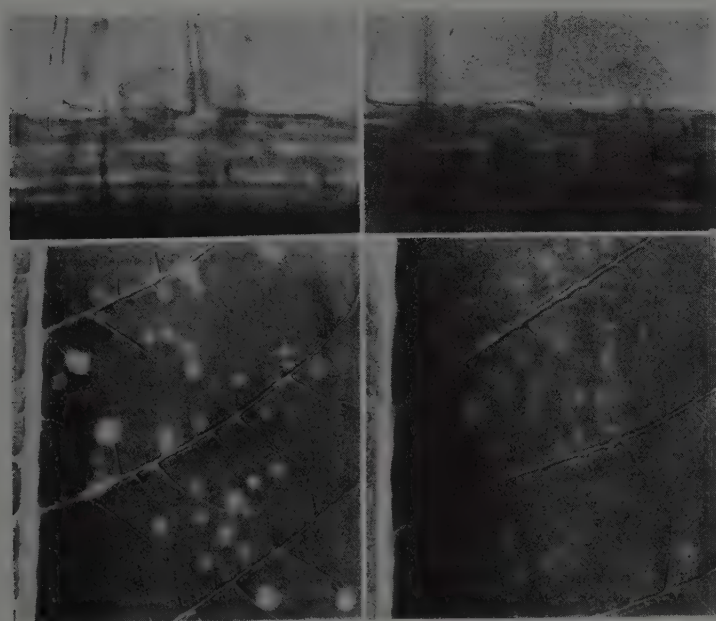


Fig. 5a.—(Upper) A 48-hour colony of *B. tabacum* on the surface of a wheat root grown from a surface sterilized seed in a damp chamber. The root was inoculated by dipping it in a dilute suspension of *B. tabacum*. (Lower) Infection on a tobacco leaf produced by crushing the above represented colony in water and pouring over the surface of the water-soaked leaf.

Fig. 5b.—(Upper) A 48-hour colony of *B. angularatum* on the surface of a wheat root grown from a surface sterilized seed in a damp chamber and inoculated with a dilute suspension of *B. angularatum* (x 200). (Lower) Infection produced on a tobacco leaf by the above represented colony crushed in water and poured over the surface of the water-soaked leaf.

Bluegrass pastures.—Tobacco plant beds in Kentucky are usually placed in a new location each year although an occasional grower uses the same site repeatedly. In central Kentucky, beds are often made along a fence in an old bluegrass pasture. In western Kentucky, beds are frequently made in clearings in a woods. Angular leafspot may be expected to develop in practically all burley beds, in plants just covering the ground, following a period of wet weather. In 1943, when there was a very wet spring, wildfire also was very prevalent in burley beds in central Kentucky. In dark tobacco, wildfire may appear in beds in nearly any location. These observations suggest that one or other of the pathogenic organisms must be almost always present in the soil.

To determine whether or not the pathogenic organisms could be isolated from bluegrass pastures, soil samples were collected with a soil sampling tube to a depth of 2 to 4 inches. These samples contained the roots of any plants which happened to be growing at the point of sampling. Usually 10 samples of 5 cores each were collected from each field. Each collection of soil was placed in a tumbler of water, stirred until the soil masses were broken up, and the liquid poured over the surface of a water-soaked leaf. Collections were made on 38 farms. No infection resulted from the collections from 18 of these but angular leafspot, wildfire, or both were obtained from collections on 20 farms. Angular leafspot alone resulted from inoculation with 15 collections (Fig. 6), wildfire alone from 2, and wildfire and angular leafspot from 3.

In one instance a plant bed was severely injured by wildfire throughout its length. Collections of grass and weed roots between the bed and the fence gave heavy infection and weed roots collected in the adjacent field, which had been plowed and prepared for setting the crop, gave heavy wildfire infection when used as inoculum. In this instance there was evidence that the plant-bed infection had come from bacteria in the soil, and proof that the bacteria were present in the field at setting time. This field had been in grass and lespedeza several years.

Method of Infection of Plants in the Bed

During periods of protracted wet weather when plants in the bed are making rapid growth, the thick, tender leaves may become water-soaked in small spots which show especially well from the under sides of the leaves. These had been observed by the writers for years but were believed to be incipient infections, but this type of water soaking may occur in beds which do not subsequently develop disease.

Braun and Johnson (10) first pointed out the relation between water soaking and plant-bed infection. If bacteria are applied to the surface of water-soaked leaves, infection takes place instantly; that is, the bacteria immediately pass into the leaf through the stomata (21). Reproduction then commences and angular leafspot or wildfire develops, depending upon which organism is present in the bed. The means by which the bacteria reach the leaf surface has not been demonstrated except that it is a common observation that the earliest wildfire spots are most likely to occur on the tips of leaves which touch the ground. Small flies are usually common in plant beds at this time and it is not unlikely that their feet become contaminated with bacteria on the surface of soil and that they carry them to the leaves. It is not uncommon for numerous angular leafspots to be

located near the tips of leaves which, because of crowding of plants, could never get very near the soil. The underside of the tips of leaves is a favorite place for the flies to rest. On small plants splashing could probably satisfactorily account for much of the infection. Heavy infections on very tender leaves frequently result in a soft rot, especially when infected with the wildfire organism.

No relation has been observed between nutrition of the plants in the bed and virulence of the outbreak except that plants low in nit-

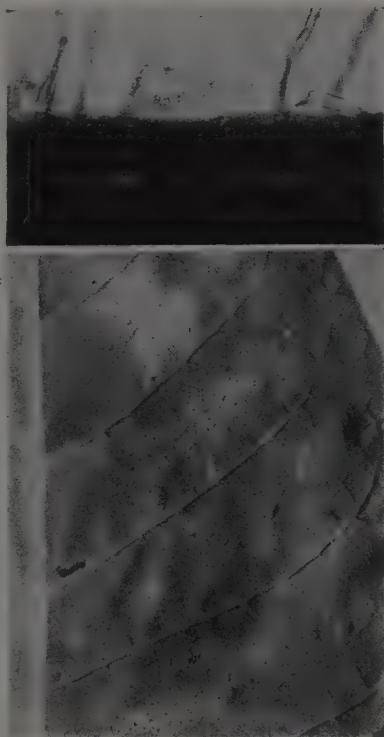


Fig. 6.—(Upper) A colony of *B. angularatum* growing on a wheat root. The organism was isolated from a leaf inoculated with a soil collection from an old bluegrass pasture. (Lower) Infection on burley tobacco from the colony shown in the upper picture.

rogen are much less likely to be severely injured than well-nourished plants. Plants which are beginning to crowd in the bed and which are approaching the setting stage are more likely to be resistant than the smaller, better-nourished plants. A high level of potash does not appear to bring about resistance in the bed although it seems to reduce injury in the field.

Control of Wildfire and Angular Leafspot in the Plant Bed

Angular leafspot is almost certain to develop in, and frequently causes considerable injury to burley plant beds following a rainy cool period when the plants are growing rapidly. It is rarely conspicuous in dark tobacco beds. During the last few years wildfire has become increasingly common in burley and dark tobacco beds although outbreaks of it are much more sporadic than those of angular leafspot. Because of the prevalence of the diseases and the serious injury which may be caused by wildfire to plants in the bed, a routine control practice in plant beds is desirable.

Applications of bordeaux mixture.—Since 1932, plant beds on the Station farm at Lexington have been treated with bordeaux mixture with excellent results as far as control of leaf-spots in the bed is concerned. The treatment was practically the same as that recommended by Beach (6); namely, two applications of 2-3-50 or 3-4-50 bordeaux mixture applied with a sprinkling pot; first, when the true leaves are just developing; and second, about 10 days later. In the early years of treatment a single application gave excellent protection to the plants, except that an occasional group of plants developed a small amount of angular leafspot, presumably in spots where bordeaux was not applied. The use of two applications has given complete control in the plant bed year after year as far as visible signs of infection are concerned. Therefore two applications are now recommended as a routine practice. In extensive field experiments and field observations in Western Kentucky and from observation and reports of growers in Central Kentucky, this treatment appears to be completely successful in protecting plants in the bed, and is now widely used by farmers.

While nothing was definitely known of the source of the bacteria in the plant bed during the time this practice was being introduced, it was clearly demonstrated that a coating of bordeaux mixture over the surface of the soil when the plants were very small gave complete control of both diseases in the bed throughout the plant-bed season. This fact emphasized the likelihood that all infection came directly from the soil and was not carried into the bed by insects or wind-

blown material after the plants had attained some size. With the knowledge that the bacteria multiply on the roots of tobacco plants in the bed, the desirability of sprinkling the bordeaux mixture directly on the soil, rather than attempting merely to cover the leaves with bordeaux applied under pressure, is emphasized.

The necessity for completely covering the plant-bed surface with bordeaux in order to get complete control has been brought out in several instances. For example, plant-beds in a location where wildfire is likely to develop have had bordeaux applied through the cotton but not to the soil around the bed. The plants in treated soil have been entirely clean, whereas plants just outside of the bed have developed heavy infection. In treated beds, where a portion has been left untreated for comparison, angular leafspot has developed to a line in the untreated portion, but even two weeks after setting, the disease had not spread into the treated portion. Therefore great care should be exercised to see that the entire surface of the bed is thoroughly covered.

It is too early to say whether bordeaux treatment will prevent multiplication of bacteria on the roots of plants in the bed and thus prevent transferring the bacteria to the field. Several tests of composite samples of roots from the bed treated at the Station in 1942 and of soil and roots treated in 1943 failed to show evidence of pathogenic bacteria on the roots. But roots from 4 of 16 farmers' beds treated with bordeaux were infected.

Field infection of plants treated in the bed.—Observations in Kentucky seem to prove that plants which were free from leafspot in the bed may become infected in the field. For 5 years every precaution was taken at the Western Kentucky Experiment Substation to raise disease-free plants in the bed. Seed was treated with silver nitrate, new or boiled canvas was used, beds were steamed, and two applications of bordeaux applied when the plants were small. The plant beds appeared to be free from leafspot at setting time. Spotted leaves were collected during the summer and inoculations made to healthy plants in the greenhouse. During the first two years angular leafspot was found to be present, and in 1935 and 1938, following a very wet spring and summer, both wildfire and angular leafspot developed to some extent. Wildfire had not been found on this farm or in that section of the state previously. Plants carried from Princeton to the Greenville Experiment Field developed angular leafspot but not wildfire. Following an excessively wet period wildfire appeared in Todd and neighboring counties in 1935, where it had not been reported before.

In a study of wildfire in Switzerland, Heirele (26) reported that wildfire was entirely absent from one district but present in others when all plants were supplied from one point, indicating that the stock was clean but that infection occurred following setting.

Extensive studies in Western Kentucky covering four seasons indicate that with dark tobacco, plant-bed treatment is definitely beneficial most years because it tends to delay the time of appearance of leafspots in the field even in very rainy seasons (29). In wet seasons bacterial leafspots have developed late in the season in fields set with healthy plants from treated beds.

On the Station farm at Lexington, angular leafspot has sometimes developed extensively in fields set with plants from beds treated twice with bordeaux which had no signs whatever of leafspot at the time of setting and for weeks after. In 1940, for example, the plant beds and crop on the Station farm were visibly free from angular leafspot up to July 20, at which time a moderately heavy rain fell for about 3 hours during the day on soil already wet. Leaves were abundantly water-soaked by 1:00 p.m. Water-soaking was apparently of the internal type as there was no wind. A few days later angular leafspot was abundant and the distribution and size of the spots corresponded very closely to the water-soaked spots. Evidently there was abundant inoculum which did not originate in leafspots. While its origin was not determined, it is probable that the roots either carried infection from the bed or acquired it from the field soil, with the result that water carrying the bacteria was splashed onto the water-soaked leaves, thus bringing about infection (45). Throughout the summer, in some years, conditions of this kind do not occur, and infection of plants, which were leafspot-free in the bed, is not likely to occur. However, weather conditions are usually such that spread of the disease occurs if there is abundant inoculum on leaves from plants which were infected in the bed.

Because of the greater prevalence of the angular leafspot organism in nature than the wildfire, it is much more likely that plant-bed treatment will control wildfire in the field than it will angular leafspot. However, there is no reason for believing that wildfire-free plants will not occasionally acquire the disease in the field after transplanting. Several instances have been observed where wildfire developed on scattered field plants for the first time as late as August 1.

Flea beetles and field infection.—Soon after tobacco is set in the field it may be attacked by flea beetles, *Epitrix parvula*. Sometimes, in place of a clean cut wound the tissues surrounding the wound turn yellow and have somewhat the appearance of angular

leafspot on an old leaf. An examination of flea beetle wounds, early in July, 1943, showed that the borders of the spots contained bacteria. When individual spots were placed in broth and used as inoculum, 24 hours later on plants in the greenhouse, abundant angular leafspot developed. In the field no spread from the infected flea beetle spots occurred higher on the plant but it was clearly evident that an abundance of inoculum had built up on the ground leaves.

Inoculation of Tobacco Leaves with *Bacterium angulatum* and *Bacterium tabacum*

Open stomata.—Infection of tobacco leaves takes place through the stomata, the minute openings on both surfaces of the leaves. The stomata are very numerous on the lower surface (about 80,000 per square inch) and perhaps one-third as numerous on the upper surface. Studies (18, 19) have shown that in general the stomata tend to close during the night and to open in the morning with sunlight. During the middle of the day the majority of stomata may close if the light is intense, if the leaf wilts, if a leaf is shaded by another leaf, or if the under side of the leaf is exposed to direct sunlight as by wind. Some stomata are open at any time of day or night. These general statements indicate the ease or difficulty of water soaking leaves with a stream of water directed against the lower surface. When the stomata are wide open, a stream of water from a syringe will immediately water-soak the portion of the leaf against which it is directed. At night it is almost impossible to bring about water-soaking except after an extended trial. Intermediate rates of water-soaking are encountered during the day, depending on the location of the leaf or the general light conditions. The ease or difficulty of water-soaking is of importance in connection with the likelihood of infection occurring during storms.

How bacteria enter the leaves.—The mechanism of entrance of the bacteria into the leaf has been studied both directly and indirectly (21). If a water suspension of bacteria is atomized gently onto the surface of leaves and the leaves are kept moist for hours, the bacteria do not enter; likewise, when leaves are kept moist for hours and then lightly atomized the bacteria do not enter. But if the leaves are atomized with some pressure, to drive water into the stomatal openings and substomatal chambers, and then are gently atomized with bacterial suspensions, infection will occur. When the leaves are water-soaked and a suspension atomized on or poured over the leaf, abundant infection will take place. When the stomata are wide open on a bright day and a suspension of bacteria is atomized on the lower sur-

face with moderate force, infection will take place, but if the same process is followed at night or after the plants have been placed in a dark room for a short time, infection will not take place or it will be extremely light (17). However, when a leaf which has water-soaked easily is immediately put in a dark room and kept long enough to close the stomata, and then a suspension of bacteria is poured over the water-soaked area, heavy infection results. But if the part not soaked is sprayed with some force with a bacterial suspension, infection will be extremely light or fail completely. The stomata in the water-soaked area either do not close with the others or, if they do close, the opening is still large enough to allow the passage of bacteria in a water column but too small to allow water to be forced in readily with an atomizer.

The conditions necessary for ready infection then are numerous open stomata, and either a bacterial suspension applied with sufficient force to drive some water through the stomata and presumably carry bacteria in at the same time, or water-soaked tissue which provides a water connection between the outside of the leaf and the inside through which the bacteria can pass. The question as to whether the bacteria swim through the stomata or are carried in in some other way has been studied. Nonmotile cocci seem to enter a water-soaked leaf as readily as motile organisms, if a suspension is poured over the surface of a water-soaked area. This has been demonstrated by surface sterilizing an inoculated leaf, after water-soaking has disappeared, and culturing the crushed tissue. Leaves inoculated with either non-motile cocci or motile organisms when cultured yield equally numerous colonies.

Diluted India ink poured over the surface of a water-soaked leaf quickly enters it. Various chemicals, as mercuric chloride and copper sulfate, which will not cause injury if poured over the surface of a normal leaf, will cause severe injury if poured over the surface of a water-soaked leaf. The chemicals may enter by diffusion but the rate of entrance seems too rapid for this relatively slow process.

Infection of Water-Soaked Leaves in the Tobacco Field

Leaves soaked by wind-driven rain.—It has long been recognized that dashing rain could cause leaf spot infection because, in a strong wind, water would be picked up from infected leaves and driven against other leaves where the bacteria could enter the stomata with water as in atomizing. Clayton (12, 14, 15) then called attention to water-soaking of leaves during storms and considered it the means of breaking down resistance of tissue to the pathogenic bacteria if the

tissues remained water-soaked for 24 hours or more. He thought water-soaking also gave an opportunity for the bacteria to penetrate the tissues rapidly and thus cause death of large areas of the leaf. The writers observed water-soaking of leaves in 1932 following driving rain, but as the water-soaking disappeared within about an hour, the water-soaked areas were recognized as areas into which the leaf-spot bacteria could be readily introduced and cause infection, but no further significance was attached to it.¹

Leaves water-soaked internally.—Johnson (31) then pointed out that another type of water-soaking could occur which he called internal. It seemed to take place during exposure of plants to a protracted period of very high humidity when the soil was wet. He stressed the importance of this condition, if it was maintained for many hours, in breaking down resistance of the leaf tissue to pathogenic as well as nonpathogenic bacteria. Braun and Johnson (10) then called attention to the close relation which they found between the amount of water-soaking and the extent of natural infection and invasion with *B. angularum* in tobacco plant beds. They mentioned the possibility of bacteria traveling for long distances in water-soaked tissue if water-soaking is maintained (3 inches in 30 hours).

Relation of water-soaking to leafspot diseases.—In the present studies it was demonstrated that water-soaked tobacco leaves, if kept water-soaked for a long time, were usually severely damaged, presumably by asphyxiation of the immersed cells, in the absence of pathogenic organisms (47). It was found further that water-soaking need not be maintained for more than the period required for the entrance of bacteria into the leaf, in order to get severe damage to inoculated leaves. These studies seemed to show that as severe damage could be caused by a few minutes water-soaking, during which time a suspension of bacteria was poured over the water-soaked tis-

¹Storm damage to tobacco leaves may be of two kinds. During dashing rains, when the under surfaces of the leaves are likely to be turned up, raindrops will cause water-soaking if the stomata are open, but if closed the raindrops may hit the leaves hard enough to bruise them, as none of the impact is taken up by water passing through the stomatal openings. In the former instance, bacteria may be carried into the leaf with the water, or a water passage opened through which the bacteria on the surface of the leaf can readily enter and cause infection, even though the leaf remains water-soaked for only a few minutes. In the second instance, where turned-over portions of leaves are bruised, the tissues are at first silvery, then gradually turn yellow and finally the bruised tissue is killed. These spots are usually free from bacteria, at least during the yellow stage. This type of injury can be reproduced experimentally by directing a stream of water against the surface of a leaf when the stomata are largely closed. Storm injury of this type is not always easily distinguished, on maturing tobacco, from the bacterial spots.

sue, as when water-soaking was maintained for several hours following inoculation.

Water-soaking appears to be much more important in affording a water channel from the outside of the leaf to the inside through which the bacteria can penetrate than in breaking down resistance to infection or as a means of spread of bacteria within the leaf after the bacteria have entered, if there is sufficient inoculum on the surface of the water-soaked leaf. When it is realized that there are about 80,000 stomata per square inch of leaf surface, through which bacteria can enter, it must be obvious that if much inoculum is present, infection could and apparently does occur all over the water-soaked area. If inoculum is very scant, it is possible that long water-soaking could play an appreciable part in increasing the extent of invasion. With high dilution of bacteria and rapid disappearance of water-soaking, discrete minute angular leafspots, apparently the result of individual infections, are scattered over the area which was water-soaked, in contrast with complete death of water-soaked tissues if bacteria are abundant.

It is not possible at present to say with certainty what parts transient water-soaking and extended water-soaking play in outbreaks of leafspot diseases in Kentucky. In two years observations made after each rainstorm, on the Experiment Station farm, several cases of storm water-soaking have been observed. In one instance, a severe wind and rainstorm occurred at 9 p.m. and water-soaking was still present the following morning but then soon disappeared. In 1940 one instance of internal water-soaking on plants about one foot tall was observed. The soil was wet from previous rains and water-soaking occurred about 1 p.m. during a gentle but steady rain. All but the growing-point leaves were abundantly spotted with water-soaked areas. Water-soaking had disappeared by about 5 p.m. A few days later the leaves were heavily spotted with large necrotic angular leafspots, the distribution and size of which were similar to the water-soaking patterns. This outbreak of angular leafspot was rather general in the county. Thus actual wind-driven rain and what is probably internal water-soaking both play a part in outbreaks of angular leafspot and wildfire.

Relative Resistance of Tobacco Leaves to Wildfire and Angular Leafspot

Numerous inoculations have been made with *B. tabacum* and *B. angulatum* on leaves of tobacco plants of all ages to determine changes which may take place in resistance during the growth of the plant.

This work was done in the hope that it might throw some light on susceptibility which develops to the blackfire type of spot as leaves age, or following topping. The results have not contributed much to this problem except to emphasize the part which potash may play in maintaining resistance throughout the ripening period. Inoculations were made with a No. 12 DeVilbiss atomizer. Twenty-four or 48-hour broth cultures were diluted with one part or more of sterile water and usually atomized upon the underside of half of a leaf and the upper side of the other half. Plants with several well-developed leaves were usually inoculated on each leaf.

Usually from the inoculation three types of reactions were observed on a plant: (1) no effect or very slight chlorosis on mature leaves; (2) distinct chlorosis with no discrete necrotic spots on leaves approaching maturity; and (3) necrotic spots surrounded by a wide halo, if wildfire, and a narrow yellow band of tissue, if angular leafspot, on leaves in an active growing condition. Necrotic spots frequently coalesced to form larger necrotic spots. The larger typically angular spots or large necrotic areas common to the field disease rarely developed, probably because water-soaking was not produced with the atomizer and consequently the infected areas were usually made up of numerous separate infections rather than nearly continuous infection, such as occurs when bacteria enter the numerous stomata of a water-soaked area.

Susceptibility of Tobacco Plants under Various Conditions to Leafspot Disease

With regard to changes of resistance of plants in the bed, Moore (36) suggested that the rather rapid subsidence of wildfire might be due to the "repressive factor" (bacteriophage) with which she worked. Braun and Johnson (10) associated outbreaks in the plant bed with water-soaking.

Burley in the plant bed.—Burley tobacco in the plant bed is relatively free from angular leafspot while the plants are small but when the leaves are thick and watery, and begin to cover the ground, until the more upright, thinner type of leaf is produced, it is highly susceptible. Inoculations were begun on May 5, 1933, in a portion of a burley tobacco bed in which plants averaged 4 leaves. The plants were about 1 inch in diameter. In three days the leaves were peppered with tiny translucent spots and soon collapsed entirely as if affected by soft rot. During the next 6 days numerous dead spots

appeared on the tips of other leaves. The method of spread was not discovered, but from the position of the spots, insects, such as small flies, were suspected. Ten days after inoculation the plants were half the size of uninoculated ones. From this time until May 18 plants were susceptible to infection. At that time the plants had 8 leaves 3' to 3½ inches long. They were only slightly susceptible and in later inoculations the plants appeared almost immune. The results of these inoculation studies correspond closely with observations of sudden outbreaks over a large territory when plants are in the susceptible stage and a rainy period ensues. After a few days of growing weather, plants rapidly recover from an outbreak of angular leafspot.

Dark tobacco in the plant bed.—Similar studies with dark tobacco inoculated with *B. angulatum* indicate that it is much more resistant to injury than burley. However, dark tobacco goes through a period identical with burley, in which the plants become highly susceptible to wildfire and then become resistant. Both burley and dark tobacco may be destroyed by a severe attack of wildfire because the plants develop systemic toxicity, the growing points become chlorotic and the plants fail to recover.

Greenhouse plants.—Plants growing in pots in the greenhouse usually have one to a few upper leaves which are highly susceptible to infection with the atomizer and develop numerous small, brown, necrotic spots. Older leaves, however, quickly become nearly immune, and develop only a yellowish chlorotic area when inoculated. The length of time leaves remain susceptible is dependent upon the vigor of growth. Lack of vigor is associated with resistance. These results conform to numerous reports that high nitrogen contributes to susceptibility of field-grown tobacco to both wildfire and angular leafspot infection during periods of rapid growth.

Plants in soil and water cultures.—Plants in water cultures in which various deficiency symptoms were produced responded similarly to those growing in soil. The same is true of plants growing in soils treated in various ways. Both in soil and water cultures plants with the most vigorous leaves were most susceptible to injury with *B. angulatum*.

Plants in the field.—Burley and dark tobacco plants growing in fertile soil in the field were inoculated with *B. tabacum* and *B. angulatum* at all stages of growth in 1932 and 1933. If due allowance is made for the longer period over which leaves remain susceptible in vigorously growing field tobacco, the results obtained correspond closely with those obtained in the greenhouse. In all

field inoculations in fertile soil, the type of spots produced was limited to the typical small necrotic angular leafspot and the typical halo wildfire spot except where numerous closely spaced infections resulted in rapid fusion of separate infections, causing limited necrotic areas. Lower leaves obviously maturing were usually resistant, rapidly growing or dark green leaves above the mature leaves were usually highly susceptible to infection, while small growing-point leaves were infected with greater difficulty.

Burley in pre-bloom and bloom.—Burley plants approaching the blooming period were found to be susceptible only in the upper few leaves while those in the middle of the plant and below were relatively resistant. Plants in bloom are still more resistant. Fig. 7 illustrates the injury to the upper and middle leaves, following inoculation of a plant 52 inches tall, with *B. tabacum*.

Topped and untopped burley.—Burley plants topped when the



Fig. 7.—Wildfire inoculation on white burley tobacco illustrating greater resistance to infection through the upper than through the under leaf surface, and greater resistance with increased age. (Left) Leaf 21 at top of a plant in bloom. (Right) Leaf 12 near middle of same plant. Left side of each inoculated from above and right from below. Inoculations with atomizer held close. Inoculated July 31, and photographed August 10, 1933.

first flowers were opening increased in susceptibility somewhat over untopped plants (8) but the leaves on the lower half of the plant were still highly resistant, although a slightly chlorotic area could be seen with transmitted light where the bacteria had entered. The uppermost susceptible leaves developed a chlorotic area with a few scattered necrotic dots after 17 days when inoculated with *B. angulatum*. Similar plants inoculated with *B. tabacum* developed necrotic spots up to $\frac{1}{2}$ inch in diameter on the upper five leaves because of coalescence of spots. Burley plants inoculated in full bloom were highly resistant to infection. Fig. 8 illustrates the degree of injury to the most susceptible upper leaf of a plant inoculated with *B. tabacum* when topped in full bloom.

Throughout the summer of 1933 inoculations duplicating those on burley tobacco were made on dark tobacco with both *B. angulatum* and *B. tabacum*. No marked differences in results were obtained from those on burley tobacco. The plants were all grown on soils of relatively high fertility.

Under and upper leaf surfaces.—Valleau (43) reported that the



Fig. 8.—Wildfire on upper, most susceptible leaf of a plant of burley topped when in full bloom showing the greater number of infections through the under (right) than through the upper surface (left), and the nearly entire absence of halos. Inoculated August 11; photographed August 17, 1933; atomizer at 4 inches.

under side of a tobacco leaf was more readily infected with *B. angulatum* and *B. tabacum* than the upper surface. Fromme and Murray (25) stated that infection occurred with equal facility through the upper and under leaf surfaces. Anderson and Chapman (4) found with *B. tabacum* that "the amount of infection has been the same, irrespective of whether the upper or lower side was inoculated." Clinton and McCormick (16), "were able to produce abundant wildfire infections on the uninjured tissues by applying the germs alone either to the upper or lower surface of the mature or nearly mature leaves. However, when they were sprayed over the immature leaves rarely

did infection occur" although the young leaves were shown to be susceptible by the prick method.

The results of inoculation studies on upper and lower surfaces may be summarized as follows. The smallest leaves of the growing point are usually fairly resistant to infection with the atomizer. The somewhat larger expanding leaves, from one to several in potted plants, depending upon their vigor, and most of the leaves of rapidly growing field plants, are highly and equally susceptible to infection through the upper and under surfaces. The leaves below the few susceptible ones on potted plants and the leaves of field-grown plants as they grow older become decidedly resistant to infection through the upper surface and more slowly resistant through the under surface (Fig. 7). The results, obtained with 13 burley plants growing in soil in gallon jars in the greenhouse, are given in Table 15. All inoculations in these and similar tests were made with a DeVilbiss nasal atomizer.

It may be concluded from these studies that tobacco plants growing on soil sufficiently fertile for rapid normal growth are susceptible to typical angular leafspot and wildfire in direct relation to succulence of the tissue, and are more susceptible to infection through the under surface when resistance begins to develop than through the upper surface. Anything which tends to increase succulence of the tissue, such as high nitrogen fertilization, rapid growth, or topping, also increases susceptibility to the pathogens.

TABLE 15.—INOCULATION WITH A CULTURE OF *B. angulatum* ON UPPER AND UNDER SURFACES OF LEAVES OF 13 BURLEY PLANTS GROWING IN SOIL IN GALLON JARS

Leaves numbered from bottom to top	Inoculated on	
	Upper surface	Under surface
1-3	$\frac{39-^a}{39}$	$\frac{42-}{42}$
4, 5	$\frac{26-}{26}$	$\frac{16-, 9+, 1++}{26}$
6, 7	$\frac{24-, 2+}{26}$	$\frac{6-, 6+, 8+++, 6++++}{26}$
8, 9	$\frac{13-, 9+, 4++}{26}$	$\frac{1-, 3+, 6++, 14++++, 2++++}{26}$
10-12	$\frac{6-, 7+, 9++, 1+++}{23}$	$\frac{2+, 1++, 8++++, 13++++}{24}$

* Denominator = Number of leaves inoculated.

Numerator = Number of leaves with a given degree of infection.

-- = no infection; +, ++, +++, +++++ = few to very numerous spots.

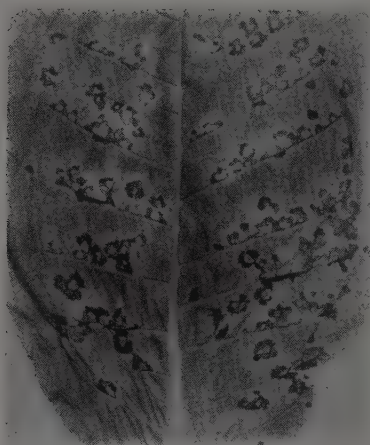


Fig. 9.—Natural infection with *B. angulatum* on white burley tobacco growing in a low-fertility area. Photographed September 6, 1935.

Untopped burley deficient in potash.—The 1935 burley variety test at Lexington was on land of moderate fertility having large spots in which available phosphorus was high but available potash was very low. In places tobacco grew normally while in other places it showed marked potash starvation. The soil areas were clearly marked by the rate of growth of plants, a row changing abruptly from large, vigorous plants to smaller, somewhat starved, plants. When the tobacco was about half grown (July 30 to August 1) a rainy period occurred followed by extensive infection of to-

bacco in this field with *B. angulatum*. The plants in the low-fertility areas were severely damaged by large angular spots (Fig. 9). Plants in areas not deficient in potash developed a light infection of typical angular leafspots similar to those produced by artificial infection on plants growing in fertile soil. There appeared to be no question, in this instance, that soil had a marked effect on the type of spot produced by natural infection.¹

On August 3, 28 comparable plants in a low-fertility area were selected to test the effect of various fertilizers on susceptibility, and the fertilizers were applied. The unaffected leaves on the fertilized and unfertilized plants were inoculated with a pure culture of *B. angulatum*, and as new leaves developed these were inoculated. In

¹In 1942 burley tobacco was grown on two adjacent plots on which alfalfa had been grown the previous two years and removed for hay. No potassium was added to one plot while a quantity equal to that estimated to have been removed in alfalfa, plus 200 pounds per acre of potassium sulfate, making a total of nearly 700 pounds of potassium sulfate to the acre, was added to the other. The plants on the no-potash plot showed marked potash starvation and were infected with angular leafspot; they were identical with those shown in Fig. 7. Angular leafspots in the high-potash plot were minute and hardly evident. Following a heavy rain on July 10 no difference in the amount of water-soaking could be seen on plants in the two plots. In both, whole leaves were sometimes completely water-soaked. Water-soaking appeared to be the external type as the rain was accompanied by a strong wind and water-soaking was on one side of the plant.

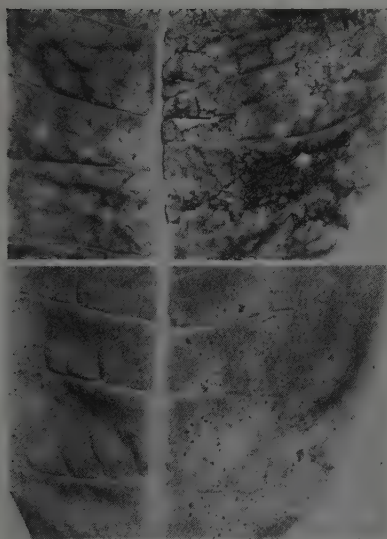


Fig. 10.— Differences in susceptibility of white burley tobacco plants in a low-fertility area after treatment with ammonium sulfate (*upper*) and potassium chloride (*lower*). Plants were inoculated on unaffected leaves with a pure culture of *B. angularum*, August 3, 1935; photographed September 24, 1935.

Fig. 10 are shown a portion of a leaf from a plant treated with potassium chloride (*lower*) and from one treated with ammonium sulfate (*upper*). The former was the least injured of the 28 plants, while the latter showed the greatest injury. The spots on this plant were fairly typical of those caused earlier by natural spread. On August 8, just after a rain, another plant in a low-fertility area where the disease was severe, was inoculated on several upper leaves with another pure culture. The photograph in Fig. 11 (*upper*) was taken 28 days later. The spots produced by inoculation were small at first but gradually increased in size, forming distinct zonations.¹

Topped burley deficient in potash.—On August 5, three plants with severe injury on the lower leaves were selected. One was topped to 15 leaves,

one to 21 leaves, and one was left untopped. Each was inoculated on half of each of the upper unaffected leaves with a pure culture of *B. angularum*. In 7 days the inoculated halves of the leaves on the two topped plants were severely injured with concentric spots typical of those which occurred naturally in this field. The spots had gradually increased in size, coalesced and killed large areas of the leaves. On the untopped plants the spots were minute or only chlorotic "clouds" marked the infected areas. The experiment was repeated on three more plants in the low-fertility area with similar results (Fig. 11, *lower*). Low topping has long been recognized as causing

¹ In 1941 the same block was used for the variety test but was fertilized with 800 pounds of 6-8-6 fertilizer per acre. There was no sign of potash starvation in the previously low-potash areas.

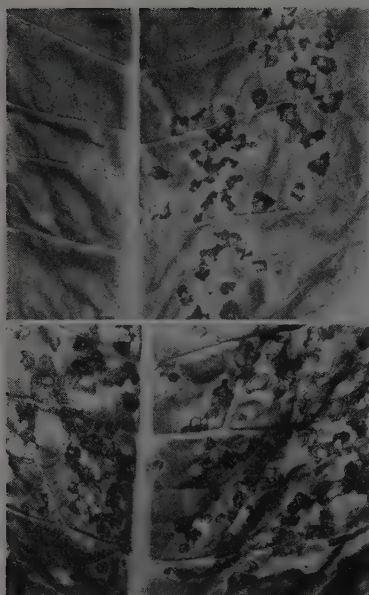


Fig. 11.—(Upper) Spots produced on white burley tobacco growing in an area low in potash by inoculation on the right half of the leaf with a pure culture of *B. angularum* on August 8, 1935, photographed September 6, 1935. (Lower) Natural infection on a leaf from a plant in a low-fertility area topped low (to 16 leaves) on August 12, 1935. This leaf (10 from below) was apparently without spots on date of topping. Photographed September 6, 1935.

greater susceptibility of dark tobacco to blackfire under some not well understood conditions.

Low, high, and untopped burley in a high-fertility area.—A similar test was conducted on 3 plants in an area where the plants grew normally and were spotted with a few typical angular leafspots. In one week spots on the low-topped plant had coalesced and killed rather large areas and finally killed the inoculated halves of the leaves. Spotting was less severe on the high-topped and untopped plants at the end of a week (Fig. 12) but even on these plants coalescence of spots finally caused large necrotic areas. The individual spots were all minute, on all 3 plants, and not of the concentric type, and with normal field infection would have been harmless.

BLACKFIRE

The nomenclature of the leafspots of tobacco is confusing both to the research

worker and to the farmer. In Kentucky, typical angular leafspot, in the plant bed and field, is called rust by the grower. Growers quickly come to recognize typical halo wildfire spots after the disease becomes established in a neighborhood. In dark tobacco the late-season concentric spot is recognized as a third disease and is called blackfire, black rust or wildfire by the grower, without respect to its cause. Late-season storm damage is also confused with this disease complex.

Experimental production.—There is some reason for thinking that three distinct diseases are concerned in the bacterial leafspot

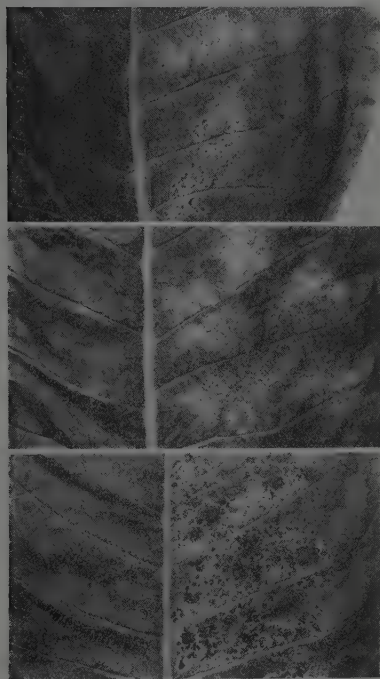


Fig. 12.—Leaves from high-topped (upper), medium, and low-topped (bottom) plants growing in a soil area where the plants seemed to grow normally. The leaves were inoculated with a pure culture of *B. angulatum* on the right half of the under side.

complex. Typical angular leafspot and wildfire are easily produced by inoculating tender, rapidly growing tobacco plants with the respective organisms, but as the leaves age they quickly become almost immune to both organisms. But it is usually after this period has been reached that the severe zonate type of spot develops (Fig. 13). While we believed that we had produced the zonate spot experimentally (30), following inoculation of old leaves with either *B. angulatum* or *B. tabacum*, by keeping the plants in high humidity, it is still possible that other factors, such as *Alternaria* as suggested by Miss McLean, of Duke University, may have been the actual cause of the zoned spots under these conditions. It still seems too early to conclude that final proof has been presented that the large zonate spots are caused directly by

either *B. angulatum* or *B. tabacum*, and even though this is finally proved to be the case the zonate spot is so distinct in appearance from the typical spots as to make it advisable to distinguish it by a different name.

Methods of control.—Although there is still a lack of complete understanding as to the exact cause of blackfire some facts known about the conditions under which it develops suggest methods for its control which are of much value to the tobacco grower. The disease is definitely related to soil fertility (27). On the soil experiment fields the disease develops later and to a less extent on plots deficient in phosphorus (and consequently relatively well supplied with



Fig. 13.—Zonate spots produced on dark fire-cured tobacco by inoculation with a tissue culture of a wildfire spot. Inoculated June 15; photographed June 30, 1938. The leaf was the fourth from below on an untopped plant about 27 inches tall. On the upper leaves typical halo wildfire spots developed. The spots illustrated are typical of the concentric spots produced in 1938 on lower leaves of untopped plants and on ripening leaves of topped and suckered plants.

potassium and nitrogen) than on plots to which phosphorus has been added. While none of the treatments on the soil experiment fields has ever given complete control, in an epiphytotic, plots to which nitrogen, phosphorus, and potassium have been added developed less of the disease than plots not receiving a complete treatment, while manured plots or those to which a liberal application of ground tobacco stalks had been added developed still less. For many years it has been pointed out in the literature of leafspot diseases that a deficiency of potassium in the soil tends to increase them, particularly those developing during ripening. Under farm conditions, it has repeatedly been observed that where manure has been used on dark tobacco leafspotting has been reduced, and where a heavy application of well-rotted manure has been applied the disease has been completely prevented. A few growers in Western Kentucky and Northern Tennessee have found that heavy applications of a medium-grade mixed fertilizer, sometimes as much as 1,500 pounds per acre, have prevented completely the development of leafspot late in the season, allowing them to leave the tobacco on the "hill" until it is thor-

oughly ripened. Use of relatively large amounts of manure and fertilizer has not only reduced leafspotting but has also greatly increased yield and quality of the crop and its appearance in the field. Most of the tobacco in areas where blackfire is severe shows very definite signs of potassium starvation. The leaves, in place of being flat and smooth, are "rim bound" and curled under and may show some of the bronzing commonly associated with potassium starvation.

In addition to soil fertility there appears to be a definite moisture relation. Blackfire usually does not develop in dry seasons but does its greatest damage in long-continued damp periods. Heavy rains do

not seem to be essential to its development because it has been observed to be severe in low areas during and following periods of heavy dew when the leaves remained wet all night and well into the day. Under these conditions a water-soaked band of tissue surrounding the spot develops each night that the leaves are wet. It will dry out the next day, leaving another concentric band of tissue.

Under moist conditions both in the field and the greenhouse, quite typical-appearing blackfire spots have followed inoculation with either the wildfire or the angular leafspot organisms. It is not clear, however, that the organisms are the direct cause of the trouble, for under drier conditions the inoculations on similar plants may have little effect. That is, at the time tobacco plants are most susceptible to blackfire injury they appear to be most resistant to infection by the pathogenic bacteria. Further study will be necessary before all of the factors concerned in the development of blackfire are understood. In the meantime control of the bacterial diseases in the plant bed and putting tobacco fields into a high state of fertility before setting the crop will largely solve the problem of preventing the blackfire type of injury in the field.

SUMMARY

1. The leafspot diseases, angular leafspot, wildfire and blackfire, continue to be destructive diseases of tobacco in Kentucky. Wildfire and angular leafspot are plant-bed diseases and diseases of rapidly growing tobacco in the field. Blackfire is a disease primarily of ripening dark tobacco or of the older leaves. A summary of studies on these diseases, made from time to time over a period of about 20 years, is reported in this bulletin.

2. *Bacterium tabacum* and *B. angulatum* die out rather rapidly in potato-dextrose (2 percent) agar. Death is preceded by an increase in hydrogen ion concentration. By reducing the dextrose content or by adding potassium hydroxide or calcium carbonate to the medium, longevity is greatly increased. Beef-peptone broth and beef-peptone agar are good media in or on which to store the organisms, as the pH remains near 7.

3. Colonies derived from a pure culture of these organisms are uniform in type on agar plates but those produced by different strains differ from one another greatly. Colonies range from watery to firm, and through varying degrees of roughness. The colonies of "rough" cultures have rather characteristic folded patterns for each colony. Rough and smooth colonies appear equally pathogenic. Colony types

are rather constant in successive transfers and passages through leaves and in storage. Individual leaf spots from plant beds yield several colony types which, when separated, are fairly constant. Pathogenicity of strains of both *B. angulatum* and *B. tabacum* ranges from faintly pathogenic to those which produce the typical spots.

4. *Bacterium tabacum* and *B. angulatum* are closely related organisms. *B. tabacum* appears to be a chlorolytic strain of *B. angulatum*. No evidence has been obtained that the organisms are identical with *Pseudomonas fluorescens*.

5. Seed is rarely infested with either organism and seed-borne organisms appear to play no part in outbreaks of the diseases. A study of hot-water treatment proved that seeds could be treated at 52° C for 20 minutes following 1 hour presoaking, while the bacteria in dried tissue were usually killed by 6 minutes exposure at 52° C.

6. The host range of the organisms is not limited to species of *Nicotiana*. They multiply as well in leaves of cowpea, soybean, pepper, groundcherry, morning-glory, tomato, jimson weed and bull nettle as in tobacco leaves and also multiply on the surface of the roots of several crop plants and weeds.

7. Outbreaks of wildfire and angular leafspot occur rather suddenly in the plant bed during cool, wet weather and frequently involve an entire bed. The causal organisms appear to be rather common soil inhabitants which live on the surface of rootlets of some weeds and crop plants. Outbreaks in the bed appear to occur after multiplication has taken place on the roots of tobacco seedlings and when weather conditions are such that the leaves become water-soaked. The organisms may be isolated all summer from the roots of infected tobacco plants growing in the field; and, all winter, from the roots of wheat or barley cover crops which follow the tobacco. Thus the organisms appear to be root-inhabiting organisms which, under certain conditions, enter the leaf of tobacco and cause a leaf-spot disease.

8. Wildfire and angular leafspot can be effectively prevented in the plant bed by one or two applications of bordeaux mixture to the surface of the soil when the plants are small. This forms a blanket of toxic material between the bacteria on the roots and the susceptible leaves. Two applications, while the plants are small, appear to give perfect control of both diseases as long as the plants are in the bed. Plants which are free from leafspot at transplanting time may contract both wildfire and angular leafspot after they are set in the field. Both *B. tabacum* and *B. angulatum* have been isolated,

though rarely, from the roots of tobacco plants from bordeaux-treated beds, indicating that infection could be carried from a treated bed to the field on roots. Infection can originate also in the field. Flea beetle injuries on the ground leaves have been found to be infected with *B. angulatum* in early July.

9. Infection of leaves usually occurs through stomata but only when the leaf tissues have become sufficiently water-soaked so that a water channel is formed from the outside of the leaf to the inside, or when bacteria are actually carried in with water dashed or sprayed against the surface of the leaf. Infection may also occur through wounds.

10. Very young leaves are not easily inoculated but all rapidly developing leaves are susceptible to both organisms. As the leaves mature they become highly resistant to infection. It is at this time that the large concentric spots, commonly called blackfire, are most likely to develop. As leaves pass the highly susceptible stage, infection takes place less readily through the upper surface than through the lower. In the plant bed, plants are more susceptible during the time the leaves are 2 to 4 inches long. As the plants get larger and begin to crowd, they become highly resistant. In the field greater injury to the leaf follows infection if the plant is growing in soil deficient in potash than if an adequate supply is available.

11. The disease known as blackfire and characterized by large zonate spots on maturing leaves frequently appears to be a continuation of an outbreak of wildfire or angular leafspot or both. The disease occurs at a time when the leaves usually do not respond to infection by the pathogenic bacteria. Further study will be necessary before a satisfactory explanation can be given for the development of the blackfire type of spot.

LITERATURE CITED

1. ALLINGTON, WILLIAM B. Observations on the epidemiology of tobacco wildfire and blackfire. *Phytopathology* 31, 957-959, 1941.
2. ANDERSON, P. J. Leafspots. Conn. (State) Agr. Expt. Sta. Bul. 367, 1935.
3. ——— Overwintering of tobacco wildfire in New England. *Phytopathology* 14, 132-139, 1924.
4. ——— and J. H. CHAPMAN. Tobacco wildfire in 1922. Mass. Agr. Expt. Sta. Bul. 213, 1923.
5. BEACH, W. S. Control of tobacco wildfire. Second report. Pa. Agr. Expt. Sta. Bul. 322, 1935.
6. ——— Investigations and control of certain leaf spot diseases of tobacco. Pa. Agr. Expt. Sta. Bul. 258, 24 1930.
7. ——— Tobacco seed bed management as related to wildfire control. Pa. Agr. Expt. Sta. Bul. 274, 1932.
8. BÖNING, KARL. Beiträge zur Kenntniss des parasitischen Verhältnisses von *Pseudomonas tabaci* Wolf et Foster, des "Wildfire" Erregers am Tabak. *Ztschr. f. Parasitenk.* 2, 645-755, 1930.
9. BRAUN, ARMIN C. A comparative study of *Bacterium tabacum* Wolf and Foster and *Bacterium angulatum* Fromme and Murray. *Phytopathology* 27, 283-304, 1937.
10. ——— and JAMES JOHNSON. Natural water-soaking and bacterial infection. *Phytopathology* 29, 2-3, 1939.
11. CHAPMAN, J. H. and P. J. ANDERSON. Tobacco wildfire. Mass. Agr. Expt. Sta. Bul. 203, 67-81, 1921.
12. CLAYTON, E. E. A new and important factor in the epidemiology of tobacco leaf diseases. Abstract in *Phytopathology* 25, 11, 1935.
13. ——— Toxin produced by *Bacterium tabacum* and its relation to host range. *Jour. Agr. Res.* 48, 411-426, 1934.
14. ——— Water-soaking of leaves in relation to development of the blackfire disease of tobacco. *Jour. Agr. Res.* 55, 883-889, 1937.
15. ——— Water-soaking of leaves in relation to development of the wildfire disease of tobacco. *Jour. Agr. Res.* 52, 239-269, 1936.
16. CLINTON, G. P. and F. A. McCORMICK. Wildfire of tobacco in Connecticut. Conn. (State) Agr. Expt. Sta. Bul. 239, 1922.
17. DIACHUN, STEPHEN. Relation of stomata to infection of tobacco leaves by *Bacterium tabacum*. *Phytopathology* 30, 268-272, 1940.
18. ——— Stomatal behavior in field tobacco. *Ky. Acad. Sci. Trans.* 9, 20-23, 1941.
19. ——— and W. D. VALLEAU. Relation of stomatal opening to water-soaking of tobacco leaves. *Amer. Jour. Bot.* 26, 347-351, 1939.
20. ——— W. D. VALLEAU, and E. M. JOHNSON. Isolation of *Bacterium angulatum* from overwintered field soil. *Phytopathology* 32, 2-3, 1942.
21. ——— Relation of moisture to invasion of tobacco leaves by *Bacterium tabacum* and *Bacterium angulatum*. *Phytopathology* 32, 379-387, 1942.

22. ELLIOTT, C. Manual of bacterial plant pathogens. Williams and Wilkins, 1930.
23. FROMME, F. D. Seed treatment for tobacco. Mimeographed sheet No. 3250, Va. Agr. Expt. Sta. 1922.
24. ——— and S. A. WINGARD. Blackfire or angular-leafspot of tobacco. Va. Agr. Expt. Sta. Tech. Bul. 25, 1922.
25. ——— and T. J. MURRAY. Angular-leafspot of tobacco, an undescribed bacterial disease. Jour. Agr. Res. 16, 219-228, 1919.
26. HEIRELE, E. Untersuchung einer unter dem Namen "Rost" in der Schweiz verbreiten Tabakkrankheit. Schweiz. Bot. Gesel. Ber. 47, 363-368, 1 fig., 1937.
27. HOPKINS, J. C. F. Blackfire of tobacco. Rhodesia Agr. Jour. 26, 371-373, 1929. Abs. in Rev. Appl. Mycol. 8, 680, 1929.
28. HOUSER, TRUE and E. E. CLAYTON. Rust of tobacco. (A physiological breakdown.) Plant diseases in Ohio for 1921. Mimeographed. Ohio Agr. Expt. Sta.
29. JOHNSON, E. M. and W. D. VALLEAU. Control of blackfire of tobacco in Western Kentucky. Ky. Agr. Expt. Sta. Bul. 399, 1940.
30. ——— STEPHEN DIACHUN, and W. D. VALLEAU. Experimental production of blackfire on tobacco. Phytopathology 30, 73-79, 1940.
31. JOHNSON, J. Relation of water-soaked tissues to infection by *Bacterium angularum* and *Bacterium tabacum* and other organisms. Jour. Agr. Res. 55, 599-618, 1937.
32. ——— and H. F. MURWIN. Experiments on the control of wildfire of tobacco. Wis. Agr. Expt. Sta. Res. Bul. 62, 1925.
33. ——— C. M. SLAGG, and H. F. MURWIN. Host plants of *Bacterium tabacum*. Phytopathology 14, 175-180, 1924.
34. KARRAKER, P. E., and C. E. BORTNER. Symptoms of phosphorus deficiency in Turkish tobacco. Amer. Soc. Agron. Jour. 25, 556-558, 1933.
35. MANIL, P. Contribution a l'etude d'une maladie bacterienne du Tabac, constatée en Belgique. Inst. Agron. et des Stas. de Rech. de Gembloux Bul. 2, 244-268, 1933. Abs. in Rev. Appl. Mycol. 13, 190-191, 1934.
36. MOORE, E. S. Studies on overwintering and dissemination of wildfire. Union of So. Africa Dept. Agr. Sci. Bul. 54, 1927.
37. ——— Wildfire of tobacco on *Nicandra physaloides*. Nature 132, 517, 1933.
38. OROS, JOAN. Desinfectarea semintei de tuton cu apă caldă. Bul. Cult. și Ferment. Tutunului. 21, nr. 2, 258-263. French summary. 1932.
39. REID, J. J., J. NAGHSKI, M. A. FARRELL, and D. E. HALEY. Occurrence and nature of the microorganism associated with wildfire. Pa. Agr. Expt. Sta. Bul. 422, 1942.
40. ——— R. G. HARRIS, and H. G. McAULIFFE. A cultural and serological study of *Phytomonas tabaca* and certain related forms. Jour. Bact. 37, 234-235, 1939.
41. STAPP, C. Bakterielle Tabakkrankheiten und ihre Erreger. Angew. Bot. 12, 241-274, 1930.
42. VALLEAU, W. D. Are blackfire and angular leafspot of tobacco identical? Abs. in Phytopathology 19, 93, 1929.

43. ——— Wildfire and angular leafspot of tobacco. Ky. Agr. Ext. Cir. 89, 1921.
44. ——— and E. M. JOHNSON. *Physalis subglabrata*: A natural host of *Bacterium angulatum*. Phytopathology 26, 388-390, 1936.
45. ——— and S. DIACHUN. Burley tobacco diseases in Central Kentucky in 1940. Pl. Dis. Rptr. 25, 1, p. 18, 1941.
46. ——— E. M. JOHNSON, and STEPHEN DIACHUN. Association of tobacco leafspot bacteria with roots of crop plants. Science 96, No. 2485, 164, 1942.
47. ——— STEPHEN DIACHUN, and E. M. JOHNSON. Injury to tobacco leaves by water-soaking. Phytopathology 29, 884-890, 1939.
48. WOLF, F. A. Wildfire of tobacco. N. C. Agr. Expt. Sta. Bul. 246, 1922.
49. ——— and A. C. FOSTER. Thermal death points of some bacterial plant pathogens in relation to reaction of the medium. N. C. Agr. Expt. Sta. Tech. Bul. 20, 1921.
50. ——— ——— Tobacco wildfire. Jour. Agr. Res. 12, 449-558-1918.
51. WSOROW, W. Infectious ryaboukha of tobacco (*Nicotiana tabacum*). Bul. North Caucasian Plant Prot. Stat., Rostoff on Don, 1930, 6-7, 261-272, 1 pl., 1930.